THE EXISTENCE OF A MICROBIOLOGICALLY INACTIVE "FOLIC ACID"-LIKE MATERIAL POSSESSING VITAMIN ACTIVITY IN THE RAT

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ONE FIGURE

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Milk has been found to contain only traces of "folic acid" (FA)[1] when assayed microbiologically following a standard digestion procedure (Cheldelin and Williams, '42). Welch and Wright ('44) have reported in a preliminary note that, although powdered whole milk does not contain significantly more FA, as determined microbiologically, than do the usual highly purified diets, the incorporation of succinylsulfathiazole (SST), in amounts up to 10% of a ration of powdered whole milk did not lead to a diet capable of producing a FA deficiency in the rat. These data were taken as evidence for the existence in powdered whole milk, and probably in other natural materials, of a component which may be utilized by the rat in lieu of material possessing the microbiological activity of FA. Since our note was submitted for publication Binkley et al. ('44) have offered evidence for the existence of a conjugate of FA (or vitamin B_c) in yeast extract that is available to the chick but not to Lactobacillus casei. FA could be released from the complex by "enzymatic digestion." Mims, Totter and Day ('44) have shown that after digestion with rat liver, according to the technique described by Wright and Welch ('43), large amounts of microbiologically active FA could be demonstrated in yeast extract and in certain other materials. Mallory, Mims, Totter and Day ('44) recently have shown that the growth-promoting and leucocytopoietic effects of yeast and liver extract were proportional to the total amount of FA found after enzymatic digestion rather than to their content of FA obtained by direct microbiological assay.

[1] In this case the term "folic acid" includes those factors, probably closely related, which promote the growth of Lactobacillus casei and Streptococcus fecalis R.

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This paper presents additional evidence for the existence of a microbiologically inactive FA-like material in milk, which possesses vitamin activity in the rat.

PROCEDURE

Weanling rats of both sexes and from two different commercial sources were used in the growth studies with powdered milk. They were caged over wide-mesh screening and fed ad libitum. The 'Klim' brand of powdered milk was used throughout. The compositions of the rations of powdered milk used in the growth studies and of the purified diets that were fed to reference animals are given in table 1.

TABLE 1

Composition of diets.

COMPONENT	DIET NUMBER					
	S-9	S-10	S-23	S-30	S-31	M-1
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	
Milk powder, 'Klim'	...	...	...	...	...	1000
Vitamin-free casein	180	180	180	180	180	....
Sucrose	618	598	598	660	610	....
'Cellu' flour	40	40	40	...	...	....
Succinylsulfathiazole	...	20	20	...	50	...

Diets M-2, M-3, M-4, M-5, and M-6 prepared by mixing 990, 980, 950, 900, and 800 gm. of M-1 with 10, 20, 50, 100, and 200 gm. respectively, of SST.

In addition to the constituents given the purified diets (S-9 through S-31) contained (per kilogram): 100 gm. Primex; 20 gm. corn oil; 40 gm. salts (Hubbell, Mendel, and Wakeman ('37)); 10 mg. 2-methyl-1, 4-naptho-hydroquinone diacetate. Diets S-23, S-30, and S-31, and M-1 contained (per kilogram): 8 mg. thiamine chloride; 16 mg. riboflavin; 40 mg. nicotinic acid; 44 mg. calcium pantothenate; and 8 mg. pyridoxine, hydrochloride. Diets S-9 and S-10 contained in addition to the above B vitamins 80 mg. inositol and 40 mg. p-aminobenzoic acid per kilogram. All the diets contained (per kilogram); 1 gm. choline chloride and 1 gm. of vitamin A, D and E concentrate compounded as follows: fish liver concentrate containing 450,000 U.S.P. units of vitamin A and 90,000 U.S.P. units of vitamin D per gm. 7 gm.; α-tocopherol 2 gm.; corn oil 41 gm.

The values which are reported for FA are expressed in terms of the presumably pure L. casei factor [2] and were determined through the use of Lactobacillus casei according to the procedure of Landy and Dicken ('42). In addition, occasional comparative assays were carried out simultaneously with Streptococcus fecalis R according to the method of Mitchell and Snell ('41).

FA was determined microbiologically in the liver samples after a preliminary enzymatic digestion. Liver samples weighing 2 to 5 gm.

[2] L. casei factor was kindly furnished by the Lederle Laboratories.

were fragmented in 20 ml. of water. An amount of takadiastase equivalent to 2% of the weight of the liver was added and digestions were carried out for 18–24 hours at 37°C. under benzene. This, of several digestion procedures, has resulted in the highest values for FA in rat liver (Wright, Skeggs and Welch, '45).

The FA content of the feces was determined on complete 24-hour samples. Blotting paper was placed beneath the cages of three animals in each group and fecal material was transferred to the refrigerator at frequent intervals throughout the day. The combined 24-hour collection of feces from each group of rats was weighed and digested in 10 volumes of 1% acetate buffer (pH 4.0) with an amount of takadiastase equivalent to 2% of the weight of the sample. Digestions were carried out under benzene for 18–24 hours at 37°C. As an inhibitor of sulfonamides 10 mg. of para-aminobenzoic acid was added to each 100 ml. of medium used for microbiological assay.

Powered milk and the purified diets were assayed for FA following digestion according to the procedure for foods described by Cheldelin et al. ('42).

RESULTS

The values tabulated below summarize the results of the microbiological determinations for the FA content of the powdered milk and highly purified diets employed: L. casei assay — milk powder rations, 0.016 μg./gm., highly purified rations, 0.010 μg./gm.; and S. fecalis R assay — milk powder rations, 0.021 μg./gm., highly purified rations, 0.00 μg./gm.

Various modifications of the Cheldelin procedure, such as the use of increased amounts of takadiastase, digestion in water, or the direct extraction by autoclaving with water or buffer have failed to increase the values for FA obtained by microbiological assay. The occurrence of only small amounts of FA in these diets required their assay at unusually high levels. In addition, certain naturally occurring purines and pyrimidines have been found to stimulate the growth of lactic acid organisms in the absence of FA. These facts tend to indicate that the values reported are probably considerably higher than the actual amount of FA present.

In figure 1 are presented the data on the growth of the animals fed the various milk diets. Accompanying the curves are the proportionate number of male and of female rats involved in the various groups. The values plotted are the averages obtained from three separate experiments. They do not include the weight changes in the animals

that failed to survive the test period or in representative animals killed for analysis. There were no deaths recorded that could be attributed to the inclusion of less than 10% SST in the diets of powdered milk. Poor growth, and the death of four of eight rats within a period of 8 weeks, resulted when a diet of powdered milk containing 20% SST was fed. At the 10% level growth was depressed but little

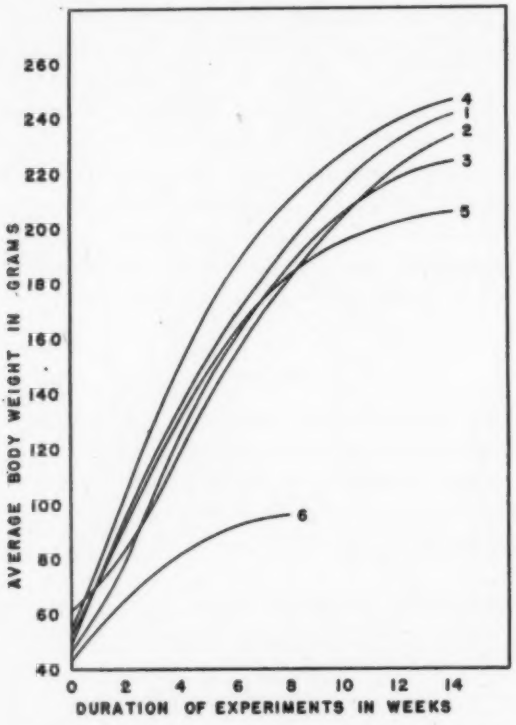

Fig. 1 The growth curves of rats ingesting various milk powder diets. (1) 0% SST, 7M, 4F; (2) 1% SST, 2M, 3F; (3) 2% SST, 2M, 3F; (4) 5% SST, 7M, 7F; (5) 10% SST, 3M, 8F; (6) 20% SST, 3M, 1F.

and only one of eleven rats failed to survive a feeding period of 14 weeks duration. Growth was entirely normal when SST was added to the diet of powdered milk in amounts of 5, 2 or 1%.

A previous study has shown that a deficiency of FA in the rat is accompanied by a low FA content in the liver (Wright and Welch, '44). After the rats had received the diet of milk powder for 5 weeks, and at intervals thereafter, determinations of FA were made on the livers

of several rats of each group. No variation was found to occur in the hepatic level of FA after the fifth week. The values given in table 2 represent group averages obtained over the remaining period of feeding. Included also are reference data for storage of FA in the livers of stock animals, of rats receiving adequate highly purified diets, and of rats receiving highly purified diets containing sufficient SST to induce a deficiency of FA. The hepatic levels of FA were obtained in the latter group when the animals had reached a plateau in growth.

TABLE 2

The "folic acid" contents of the livers of rats receiving various diets.

TYPE OF DIET	LEVEL OF SUCCINYL-SULFATHIAZOLE	CONDITION OF ANIMALS	"FOLIC ACID" [1] CONTENT		
	%		μg./gm.		
Stock	0	good	9.7	(8.4–12.0)	(3)
Purified (S-9)	0	good	1.7	(0.79–4.0)	(4)
Purified (S-10, S-23)	2	deficient	0.40	(0.13–0.73)	(10)
Purified (S-31)	5	deficient	0.30	(0.20–0.35)	(6)
Milk powder (M-1)	0	good	3.3	(1.6–6.4)	(8)
Milk powder (M-2)	1	good	2.9	(1.2–5.3)	(4)
Milk powder (M-3)	2	good	1.8	(1.3–2.9)	(4)
Milk powder (M-4)	5	good	0.95	(0.45–1.6)	(10)
Milk powder (M-5)	10	good (?)	0.45	(0.30–0.68)	(10)
Milk powder (M-6)	20	deficient	0.28	(0.17–0.44)	(6)

[1] The results given are in terms of the fresh weight of the liver employed. Accompanying the average values presented are (1) the range in values obtained, and (2) the number of liver samples studied.

A level of approximately 0.5 μg. (or less) of FA per gram of liver has been found to be associated with a cessation of growth and with other evidences of a deficiency of FA. The inclusion of 2% SST in highly purified diets readily induced such low hepatic levels. In contrast, it was necessary to incorporate from 10 to 20% SST into a powdered milk diet in order to obtain such a correspondingly low level of FA in the liver.

The effect on the fecal elimination of FA of feeding a diet of powdered milk, as compared with other diets, is shown in table 3. The inclusion of large amounts of SST in the milk diets decreased the amount of FA eliminated daily in the feces. However, the incorporation of as much as 20% SST in the powdered milk diets did not decrease the fecal elimination to a value comparable to that produced by the inclusion of 2% SST in a highly purified diet. It appears from the data in table 3 that, while there is a relationship between the elimination

of FA in the feces and the occurrence of signs of deficiency, there is no critical level of FA elimination below which deficiency signs definitely will occur.

TABLE 3

The "folic acid" contents of feces of rats receiving various diets.

TYPE OF DIET	LEVEL OF SUCCINYL-SULFATHIAZOLE	CONDITION OF ANIMALS	"FOLIC ACID" [1]		
	%		μg./rat/day		
Stock	0	good	49	(34–74)	(6)
Purified (S-30)	0	good	2.1	(1.5–2.6)	(2)
Purified (S-23)	2	deficient	0.51	(0.43–0.60)	(3)
Milk powder (M-1)	0	good	6.8	(2.2–20.8)	(7)
Milk powder (M-4)	5	good	1.4	(0.79–2.6)	(5)
Milk powder (M-5)	10	good (?)	2.0	(1.0–3.6)	(5)
Milk powder (M-6)	20	deficient	3.9	(1.4–6.5)	(2)

[1] Accompanying the average values presented are (1) the range in values obtained, and (2) the number of assays performed.

Representative animals from the control and from each sulfonamide-fed group were autopsied at the conclusion of the experiment. The histological changes encountered were for the most part limited to lesions in the kidneys, ureters and bladder produced by the deposition of concretions of sulfonamide. Concretions were encountered in two of three animals examined in the 5% group (14 weeks of feeding), in all of three animals in the 10% group (14 weeks) and in five of six animals in the 20% group (8 weeks). In only one animal of six in the 20% SST group was there encountered hyperplasia of the thyroid, as described by Daft et al. ('42), Mackenzie and Mackenzie ('43) and Astwood et al. ('43). Mild involutional changes of the thyroid were encountered in the three animals examined in the 10% group. In none of the animals were there observed any lesions of the myocardium, vascular system or liver such as were described by Daft et al. ('42).

At the conclusion of the experiments in which dried milk and SST were fed, the total and differential leucocyte counts of representative animals were determined. The results are summarized in table 4. Accompanying these data are the results of similar determinations performed with normal stock animals. For brevity, only the percentage of neutrophils in the differential count is given. Rats which had been fed 20% SST in rations of powdered milk were found to have a definitely lowered total leucocyte count and a decreased percentage of circulating neutrophils. In agreement with the previous data on growth and on hepatic storage, the animals given 10% SST might be classed as "borderline" with reference to the possible existence of a

deficiency of FA. These animals were found to have a somewhat lowered total count although the percentage of circulating neutrophils was normal. There was no evidence of blood dyscrasia in the animals ingesting less than 10% SST in diets of powdered milk for as long as 14 weeks. Occasional bacterial counts (Strawinski, Verwey and Munder, '45) were made on the feces of rats fed the powdered milk diets. The fecal flora, with respect to number and kind of bacteria, was similar to that of rats fed highly purified diets of comparable sulfonamide content.

TABLE 4

The total leucocyte counts and percentages of neutrophils in the blood of rats receiving various diets.

TYPE OF DIET	LEVEL OF SUCCINYL-SULFATHIAZOLE	CONDITION OF ANIMALS	DURATION OF FEEDING	TOTAL COUNT [1]	NEUTROPHILS [1]
	%		*weeks*	*thousand/cmm.*	%
Stock	0	good	..	13.0 (8.9–17.2) (16)	23.6 (13.7–35.0) (16)
Milk powder	0	good	14	10.8 (6.5–16.4) (4)	.....
Milk powder	1	good	14	11.5 (7.6–15.5) (2)	.....
Milk powder	2	good	14	14.7 (8.5–21.0) (2)	.....
Milk powder	5	good	14	12.8 (4.6–21.7) (5)	15.3 (10.5–23.0) (5)
Milk powder	10	good (†)	14	6.3 (3.3–9.0) (6)	19.2 (15.0–25.0) (6)
Milk powder	20	deficient	8	6.3 (3.2–8.9) (3)	9.6 (5.0–18.5) (4)

[1] Accompanying the average values presented are (1) the range in values obtained, and (2) the number of determinations performed.

DISCUSSION

Data summarized in the foregoing sections have supplied considerable evidence that diets of powdered milk, although extraordinarily low in FA as usually determined microbiologically, have a marked capacity to protect the rat against the development of a deficiency of FA, as is produced by small amounts of SST in highly purified diets.

It has been demonstrated that critically low hepatic levels of FA could be produced by adding large amounts of SST to diets of powdered milk. The fecal elimination of FA remained relatively high despite the large amount of SST in the diet. This would indicate either that the utilization of FA may have been impaired by the addition of the

drug to the diet or that increased intestinal synthesis of FA was not a primary factor in protecting the rat against FA deficiency produced by the drug.

We have attempted to utilize various techniques in vitro to supplement the conclusions derived from animal feeding experiments. Studies on rat liver, incubating in vitro, have indicated the existence in milk of significant amounts of "potential FA"; that is, a substance which could be measured microbiologically after the action of liver enzymes (Wright and Welch, '43). Since the "observed FA" content of rat liver may be modified by many factors (Wright, Skeggs and Welch, '45) it is difficult to assign more than qualitative significance to the results of these incubation studies. The use of purified preparations of the enzyme in rat liver capable of "activating" the non-microbiologically active "potential FA" is not subject to these limitations (Mims, Totter and Day, '44). Through the kind cooperation of Dr. Totter samples of the powdered milk used in our diets have been assayed for FA following incubation with the partially purified enzyme. Dr. Totter has reported to us that samples of powdered milk contain 0.48 to 0.60 μg. of FA per gram after such digestion (while before digestion only 0.016 to 0.021 μg. could be found).

If a growing rat may be assumed to ingest 10 gm. of the diet of powdered milk per day, such consumption would supply at the most a dietary intake of only 0.2 μg. of microbiologically active FA. Although quantitative data for the dietary requirement of the rat for FA have not as yet appeared, the ease with which a deficiency of FA may be produced with purified diets containing comparable amounts of FA and only 0.5 to 2% of SST would indicate that the daily requirement of FA exceeds 0.2 μg. Also, it has been shown (Welch and Wright, '43) that 5 to 6 μg. of FA per day, in conjunction with biotin, will cure the signs of deficiency of FA induced by the ingestion of highly purified diets containing 2% SST. Since a rat consuming approximately 10 gm. of the diet of powdered milk per day ingests 5–6 μg. of "potential FA," it seems justifiable to conclude that bovine milk contains a naturally occurring form of FA which serves in lieu of or as a source of FA in the metabolism of the rat, but which is not utilizable as such by the lactic acid bacteria commonly employed in microbiological assays.

SUMMARY

1. In order to produce the syndrome of "folic acid" (FA) deficiency in the rat it is necessary to include much larger amounts

of succinylsulfathiazole (SST) in diets of powdered milk than in highly purified diets of comparable FA content.

2. The deficiency resulting from the feeding of diets composed of powdered milk and large amounts (10 or 20%) of SST is characterized by a failure in growth, the attainment of low hepatic levels of FA, a low total leucocyte count, and a low percentage of circulating granulocytes.

3. Large amounts of FA are eliminated in the feces of rats fed exclusively on powdered milk. This fecal elimination of FA is reduced by feeding SST, but a syndrome of FA deficiency can exist in the presence of a considerable fecal elimination of the vitamin.

4. By the use of liver digestion techniques or by the use of a more highly purified enzyme capable of releasing FA from a non-microbiologically active complex, it was possible to show that powdered milk contains significant amounts of "potential FA."

5. It is concluded that the "potential FA" of milk, unavailable as such to microorganisms, is utilizable by the rat. The existence of such "potential FA" offers an explanation for the apparent discrepancy between the FA content of milk powder found microbiologically and that indicated by the results of growth experiments in rats.

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MITOTIC ACTIVITY AND WOUND HEALING IN THE CORNEAL EPITHELIUM OF VITAMIN A DEFICIENT RATS[1]

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ONE FIGURE

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The present study is one of a series directed toward discovering factors which control the mitotic and wound healing activity of the corneal epithelium (Buschke, Friedenwald and Fleischmann, '43; Friedenwald and Buschke, '44a, '44b). Since vitamin A deficiency gravely affects this tissue, we were interested to find out whether and in what way the activities which we were studying were influenced by such deficiency. Presumably because of the general retardation of body growth, Rosenberg ('42), has inferred that "Vitamin A can be regarded as a stimulus to the building of new cells." On the other hand, Wolbach and Bessey ('42) and Wolbach and Howe ('25, '33) state that in vitamin A deficient tissues "the rate of growth is rapid as attested by numerous mitoses of the basal cells," and they conclude that the growth activity of the epithelium is greatly augmented in vitamin A deficiency.

In view of this contradictory state of affairs, it would be desirable to obtain quantitative data on the "growth activity" of tissues in vitamin A deficiency. Attempts in this direction have heretofore been made mainly in regard to the healing time of larger wounds (Arey, '36 rev.; Buschke, '36 rev.; Heinsius, '37; de Roetth, '40). The methods employed in those studies, however, do not permit any differentiation between mitotic activity, non-mitotic cell movements, and — in at least some of the earlier studies — possible beneficial effects of vitamin A on secondary infections. It is perhaps due to this complexity of experimental factors, that the results obtained in various laboratories were not in agreement. In the present paper we have used methods of study which

[1] This work was supported in part by a grant from the John and Mary Markle Foundation.

make it possible to investigate the effects of the deficiency on mitotic activity and on cell movements in wound healing separately.

In view of the normal variation of mitotic activity from animal to animal, the experiments were performed on a sufficiently large group to make possible valid statistical analyses of the results. Seventy-four rats were used in this study to estimate mitotic activity and post-traumatic cell movements, thirty-six vitamin A deficient and thirty-eight control rats. In addition, about 1 dozen animals kept on the A-deficient diets, developed severe corneal lesions typical of the vitamin A deficiency and were no longer amenable to a quantitative interpretation on the two cell functions under investigation. These latter animals served as qualitative guides and controls for the time course in our feeding experiments.

METHODS

The feeding experiment. Five groups of 3–4 weeks old albino and hooded rats of between 25 and 50 gm. weight were put on the experimental diets. The feeding time extended over a period of from 5 to 9 weeks, and the experiment was conducted during the months of January to July.

The diet used in four of these five groups was identical with that described by Sullivan and Evans ('42) in their "experiment I".[2] In this diet the vitamin A supplement for the control animals is supplied in fish liver oil which contains vitamin D in addition to vitamin A. One experiment was carried out without this extra vitamin D through supplying the vitamin A supplement for the controls in the form of carotene in oil.[3] Since the results in this group were essentially the same as those in the four other groups, our final statistics include all five groups without differentiation. The animals were kept in individual feeding cages with raised wire net bottoms. The rats were weighed once a week. The food intake of the deficient rats did not differ significantly from that in the control groups except in the latest stages of the deficiency.

The histological methods. The eyes were enucleated under ether anaesthesia, care being taken that the animals had not been excited for at least 2 hours preceding enucleation (Friedenwald and Buschke, '44b). The eyes were fixed in formalin-alcohol, and flat preparations of the corneas were made as previously described (Buschke, Friedenwald and Fleischmann, '43).

[2] The alpha-tocopherol used as vitamin E supplement was kindly supplied by Merck and Co., Rahway, N. J.

[3] SMA CO.

The methods of assay. The methods of assay of the number and rate of mitoses have been previously described (Buschke et al., '43). The mitotic rate was determined by mitosis counts in rats who had been injected with 5 mg. colchicine per kilogram body weight 4 hours prior to enucleation. These counts as well as those with colchicine were made on flat preparations of the corneas.

The method of assay for post-traumatic cell movements in the rat's corneal epithelium has been described elsewhere (Friedenwald and Buschke, '44a). In the present experiments, the eyes were removed for histological study 3 hours following the injury.

RESULTS

The animals were sacrificed and enucleations were made when the deficient rats had stopped gaining or began losing weight. This varied from 5 to 9 weeks after institution of the experimental diets. At this time some of the animals in each series showed some other manifestations of the deficiency: Bloody exudate from the nose, blood stain of the tails, alopecia of the lids, extensive depigmentation of the incisor teeth, conjunctivitis, xerosis of the conjunctiva, and in some cases, beginning epithelial irregularities and slight opacification of the cornea. Animals which showed more severe changes of the corneas were not included in the quantitative study. Much to our surprise, the results on mitotic activity which will be statistically evaluated below, did not bear out any quantitative parallelism between the presence and severity of the symptoms enumerated here and the mitotic activity in individual cases. Therefore, no useful purpose would be served here by giving detailed descriptions or individual "case histories" of the progress of the deficiency. There was, however, a statistically significant difference between the mitotic activity in the deficient animals on the one hand, and that of the control animals on the other hand.

I. Mitosis counts

Mitosis counts in a meridional strip 0.11 mm. in width and crossing the whole cornea ranged from 2 to 142 with an average of 70, in twenty eyes of the deficient animals. In the control rats mitosis counts on twenty-seven eyes ranged from 75 to 206 with an average of 106. The critical ratio [4] of the means of these two groups is 3.2, and the difference therefore can be considered as statistically significant.

[4] Critical ratio $= \dfrac{m_1 - m_2}{\sqrt{\sigma_{m_1}^2 + \sigma_{m_2}^2}}$

The interpretation of this finding is, however, not directly apparent. In connection with the general arrest of growth the length of the corneal strip was somewhat less in the deficient rats than in the controls. In no case did the difference in corneal diameters exceed 20% and the average difference was less than this. It may be concluded, therefore, that the number of mitoses per square millimeter of tissue was significantly less in the deficient than in the control animals. If, however, it is desired to compute the mitosis rates not in terms of tissue area but in proportion to the total number of cells present a further correction has to be made. As will be pointed out below the epithelial cells in the corneas of the deficient rats are often larger than those in the controls. In extreme cases the cells may be as much as twice as large in the deficient, consequently the number of cells is on the average less in the deficient than in the controls. Counts of the number of cells in the basal layer of the corneal epithelium were made on six deficient and six control eyes. These counts were made on the same meridional strips 0.11 mm. in width which had previously been used for mitosis counts. The specimens selected for the basal cell counts were chosen from those that showed the high, low, and medium numbers in the mitosis counts and were therefore representative of the average and of the range of variation in mitosis counts for the deficient and for the control group respectively. In the six corneas of deficient animals the mitosis counts ranged from 2 to 142 with an average of 71. The basal cell counts ranged from 5,676 to 12,024 with an average of 9,231. In the six corneas of control rats the mitosis counts ranged from 76 to 206 with an average of 116. The basal cell counts ranged from 10,968 to 13,194 with an average of 12,170. Thus there were approximately 25% fewer cells in the basal layer per strip in the deficient than in the controls. Consequently the number of mitosis per 1000 basal cells is not significantly reduced in the deficient animals, though the number of mitoses per square millimeter is significantly reduced.

II. Mitotic rate

As has been pointed out elsewhere, the absolute number of mitoses present in a certain area or per thousand basal cells, does not provide a complete answer as to the mitotic activity of the tissue. A relatively low number of mitoses may be found either with a decreased rate of onset (mitotic rate) or with an increased speed of the mitotic cycle. The mitotic rate can, however, be gauged with colchicine which leads to an accumulation in metaphase of all mitoses which have entered the mitotic cycle during a given period of time (Buschke, Friedenwald, and Fleischmann, '43).

Counts were made with our method 4 hours after an intramuscular injection of colchicine, on the corneal mitoses in thirty deficient and thirty-six control animals. In the deficient group the counts varied from 50 to 344 with an average of 155. In the control rats figures of between 148 and 890 were found with an average of 335. The critical ratio of the means of these two series of data is 7, and the difference, therefore, can be considered as certainly significant, in spite of the wide scattering of the extreme values. These figures refer to the mitosis rates per square millimeter. If correction is made for the fact that the number of basal cells is less in the deficient animals the mitosis rate per 1000 basal cells is found to be approximately 30% less in the deficient than in the controls. Allowing for the standard deviations in the basal cell counts this figure is still statistically significant.

Thus a decrease of the overall mitotic rate of about 30% is found in the vitamin A deficient animals, or — neglecting the duration of the mitotic cycle itself — a prolongation of the intermitotic rest period by approximately 40%.

III. Speed of mitotic cycle

The mitotic rate per 1000 basal cells as estimated from the colchicine experiments was reduced in the deficient rats by 30%, but the number of mitoses computed on the same basis found in the counts without colchicine was not significantly reduced. The significance of this can be seen as follows. If the duration of the mitotic cycle were normal, the mitosis counts without colchicine should show the same percent decrease as in the counts with colchicine. On the other hand, if the mitotic cycle were slowed down in the same proportion as is the rate of entrance into mitosis, then the mitosis counts without colchicine should show no difference in the deficient rats as compared with the controls. It is evident from this that the mitotic cycle is somewhat slowed down in vitamin A deficiency in approximately the same degree as is the rate of onset of mitosis.

IV. Individual mitotic phases

More detailed information about the speed of the individual phases of the mitotic cycle may be obtained by determining the phase distribution (Buschke, Friedenwald, and Fleischmann, '43).

The individual phases of mitosis (prophase, metaphase, anaphase, telophase, reconstruction phase) per hundred mitoses were determined in fourteen corneas of A-deficient rats and in seventeen corneas of control rats. No significant difference between these two groups was found

in respect to the phase distribution. It can be concluded that the slowing of the mitotic cycle described in the previous section extends uniformly over the whole mitotic cycle and does not affect any phase predominantly, once mitosis has gotten under way. The effect is thus fundamentally different from the action of colchicine which arrests mitosis in metaphase.

V. Attempts at restitution from mitosis inhibition

For methodical reasons, it is difficult to test for the reversibility of the mitosis inhibition in vitamin A deficiency: As has been shown in section *I* and *II*, the inhibitory effect need not be manifest in any individual case, although statistically the phenomenon can be shown to exist. However, in one case where the number of mitoses in an ordinary count was down to 2 in one eye and where also some qualitative histological changes (see below, section *VI*) were found, vitamin A was supplemented; 9 days later the number of mitoses was counted in the second eye and found to be 110.

Similar experiments cannot be carried out in regard to the mitotic rate, as tested with colchicine, because the dose of this drug used in these experiments is fatal in the majority of animals after 12–24 hours.

We have attempted also to test the effect of supravital application of vitamin A on the mitotic rate following enucleation of the eyes of A-deficient colchicine injected animals. The results are not conclusive.

VI. Post-traumatic cell movements

Following superficial epithelial injuries, the cells of the corneal epithelium undergo characteristic changes in shape, orientation and position which may easily be seen on stained flat preparations (Friedenwald et al., '44a). These cell movements lead to the covering of small defects of about 30 microns diameter within a period of about 3 hours without the participation of mitosis.

Small epithelial injuries (needle pricks) were produced in the corneal epithelium of twelve eyes of A-deficient rats and in eleven eyes of control rats. Three hours later the eyes were enucleated and fixed for histological study. Only one of these twelve showed a significant inhibition of the epithelial cell movement. Several of the other eyes which failed to show any inhibition of the wound healing cell movements, did show some qualitative signs of A deficiency in the corneal epithelium. All of the control eyes showed normal post-traumatic cell movements.

VII. Histologic observations

In view of the excellent descriptions of the histopathology of A-deficiency in the cornea (Wolbach et al. '25, '33, '42; Goldblatt and Benischek, '27), it will be sufficient here to describe briefly merely one feature which is particularly apparent in stained flat preparations. A peculiar feature in some flat preparations of A-deficient corneas is the enlargement of the horizontal diameter of the basal layer of cells and of their nuclei to as much as twice their normal size (fig. 1). As noted

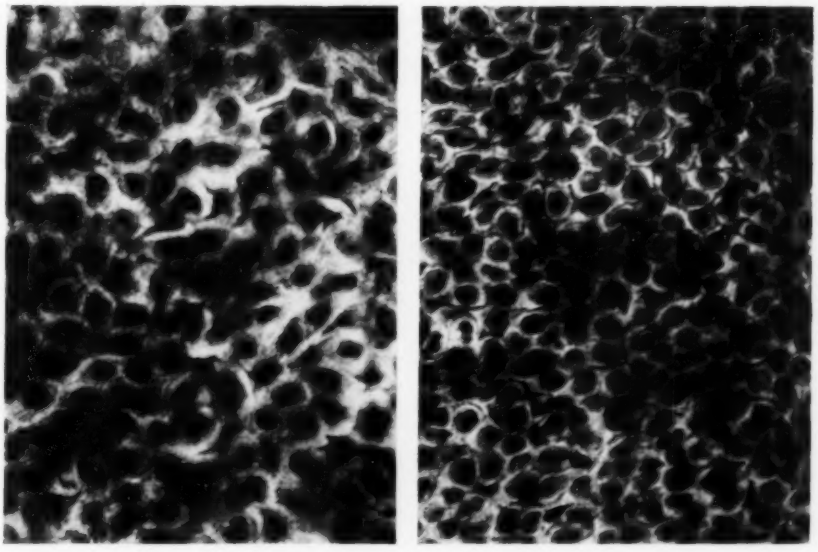

Figure 1

A B

Corneal epithelium of A-deficient rat Corneal epithelium of control rat

Flat Preparations Harris' Hematoxylin

above the deficient animals showed on the average 25% fewer cells in the basal layer of the corneal epithelium than did the normal. Between 10% and 15% of this decline is to be attributed to the fact that their corneal diameters are slightly smaller. The remaining discrepancy is a measure of the average increase in cell size. This measure, however, refers only to the relative sizes of the cells in their horizontal dimensions. In order to estimate the average cell volume it is necessary to know also the altitude of the cells. For this purpose sagittal histological

sections were made of several eyes which showed an average or an extreme horizontal enlargement of the cells and these were compared with corresponding samples from among the controls. The vertical diameter of the cells of the basal layer of epithelium was found to be about 10% less in A-deficient rats than in the control rats; since the horizontal diameters were on an average about 15% larger in the A-deficient group, the cell volume of the basal epithelial layer is somewhat larger in this group as compared with the control group. The decreased rate of mitosis cannot, therefore, be attributed to a failure of the growth of the individual cells. Some of the large cells are markedly elongated.

DISCUSSION

Our experiments show that there is a profound, though variable inhibition of mitotic activity in the corneal epithelium of vitamin A deficient rats. Whether the variations in the inhibition represent cyclic variations in the individual rat or variations from rat to rat we cannot say. What is perhaps significant is that the severity of the inhibition was not found to be correlated with the severity of other grosser symptoms of vitamin A deficiency. As to the mechanism of the inhibition, no positive conclusions can be drawn at the present time. It may be pointed out, however, that among a large number of experimental inhibitors of mitosis which we have so far studied, only two others resemble vitamin A deficiency in inducing a simultaneous decline in the rate of entrance into mitosis, and in the rate of progress through the mitotic cycle. These two other inhibitors are barbiturates and the effect of removing the superior cervical sympathetic ganglion (Friedenwald et al. '44b). No suggestions can be made as to a common denominator among these three very dissimilar experimental conditions.

The recent literature contains a number of reports (Tansley, '36; Johnson '39, '43; Hale '33, '35) on the production of a variety of developmental anomalies in vitamin A deficient embryos and young animals. In view of our findings, it would seem possible that these anomalies may result from deficient mitotic activity in certain tissues.

In contrast to the marked inhibition of mitosis produced by vitamin A deficiency in the corneal epithelium, we have failed to find any marked reduction of those non-mitotic cell movements which are concerned in the healing of small wounds of the corneal epithelium. This contrast perhaps accounts for some of the disagreements among previous investigators in respect to the influence of vitamin A deficiency on growth and repair.

SUMMARY

1. The mitotic activity and the cell movements in wound healing have been examined in the corneal epithelium of vitamin A deficient rats.

2. The overall mitotic rate per 1000 basal cells was found to be reduced by about 30% in vitamin A deficiency.

3. The speed of the mitotic cycle was likewise found to be similarly reduced.

4. The epithelial cells in some vitamin A deficient animals were found to be considerably enlarged in their horizontal diameters. Consequently the inhibition of mitosis cannot be attributed to a failure in the growth of the individual cells.

5. The post-traumatic cell movements in the healing of small wounds in the corneal epithelium were not found to be significantly delayed.

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STUDIES ON THE COMPARATIVE NUTRITIVE VALUE OF FATS

VI. GROWTH AND REPRODUCTION OVER TEN GENERATIONS ON SHERMAN DIET B WHERE BUTTERFAT WAS REPLACED BY A MARGARINE FAT [1]

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TWO FIGURES

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In the recent paper of Boutwell, Geyer, Elvehjem, and Hart ('43), it was reported that growth was as satisfactory when various oleomargarines were incorporated in the diet as when butter was employed where the main carbohydrates were glucose, sucrose, dextrin, starch or a mixture of them. These authors state, however, that a final answer can only be obtained if similar results can be demonstrated on experiments in which growth to maturity and reproduction are studied. The present experiments which have been continued over 4½ years would seem to offer such proof.

Sherman and Campbell ('37) point out that diets which are nutritionally adequate may not, however, be optimum. An example of such an adequate diet is afforded by their diet A which had supported growth and reproduction for forty generations according to their last report, at which time the animals were still thriving. When the whole milk powder in this diet was increased from one-sixth (diet A) to one-third (diet B), a marked improvement in a number of biometric measurements which are generally regarded as indices of the nutritive value of a diet resulted (Sherman and Campbell, '24). Thus, the rats were larger at weaning, they grew at a faster rate and were larger at all ages, the duration during which reproduction was possible in the females was practically doubled, and an increased number of rats was reared. Also, a larger

[1] This work was carried out under a research grant from The Best Foods, Inc. The authors wish to acknowledge the helpful advice of Prof. Anton J. Carlson of the University of Chicago, of Prof. Arthur W. Thomas of Columbia University, and of Dr. H. W. Vahlteich of The Best Foods, Inc., during the course of the experiments.

proportion of the rats were born and successfully raised through weaning on diet B than on diet A, the total failures being reduced from 48 to 19%. In a later study, it was also found that the span of life was prolonged by a statistically significant period in those rats which received diet B (Sherman and Campbell, '29–'30). These authors have concluded more recently (Sherman and Campbell, '37) that the superior nutritive value of diet B is to be ascribed to the increased content of calcium, vitamin A and riboflavin that it contains. The action of these factors is independent. Butterfat, for example, actually slows down the rate of growth, both of males and females, although its addition results in an extension of adult vitality and an increase in the life span with eventually a higher body weight being reached. However, the age at which females bore their first young was not decreased by the addition of butterfat to diet A.

The present experiments were designed to determine whether the superiority of diet B is to be ascribed to the butterfat itself or merely to the fact that this component in the diet is the vehicle whereby an adequate quantity of vitamin A is assured. Earlier results from this laboratory (Deuel et al., '44 a, '45) have indicated that butterfat per se is not required for the growth of rats but that just as satisfactory results are obtained when it is replaced by corn, cottonseed, olive, peanut or soybean oil, or a margarine on a diet in which adequate amounts of the fat-soluble vitamins are supplied. It was also found that diets containing the above fats were equally satisfactory for pregnancy and lactation in the rat (Deuel et al., '44 b). However, these studies were made on only one generation; in the present study it was desired to determine whether a diet as Sherman outlines will prove adequate over a number of generations when the butterfat is replaced by a vegetable fat to which are added sufficient amounts of the fat-soluble vitamins. The fat chosen was from a commercial vegetable fat margarine purchased on the open market which is fortified with vitamin A.[2] Adequate quantities of vitamins D and E were present in the fat.

EXPERIMENTAL

The diet employed was similar to diet B used by Sherman and Campbell except that a margarine fat was employed in place of butterfat. Its composition is given in table 1.

[2] The margarine used for the first two generations had a declared vitamin A potency of not less than 7500 I.U. per pound and that subsequently employed not less than 9000 I.U. per pound. On the basis of bioassay determinations made in our laboratory, we can state that the earlier samples of this margarine contained approximately 12,000 I. U. per pound and the subsequent ones used for the last eight generations contained approximately 15,000 I.U. per pound.

In addition to this diet, each rat received 5 gm. of lean meat and of lettuce once weekly after weaning. Campbell and Sherman ('38) have found that the feeding of fresh meat and green beans three times weekly to rats fed their diet B to some extent further improved the diet.

Twenty-one day weanling rats from our stock colony were started on the modified Sherman-Campbell diet in April, 1940. These are listed in the figures as generation 1. They were bred when they reached 3 months of age through the fifth generation after which they were mated when 4 months old. The group listed as first litter rats were the descendents of the original group through the first litters. The average age of the females when their litters were born was between approximately 110

TABLE 1

Diet 57 — Modified Sherman Diet.

INGREDIENTS		COMMENT
	%	
Skimmed milk power	23.76	Challenge brand. Contained approximately 1.10% of lipid.
Margarine fat	9.24	This proportion of added margarine fat gives the fat content equivalent to that which would be present if 33% of whole milk powder (containing 28% fat) were used.
Ground whole wheat	66.00	The wheat was ground as needed, using a Hobart mill.
Sodium chloride	1.0	

and 120 days through the fifth generation, and 150 days in the later generations. The series marked "second litter rats" were continued through the second litter of the second litters of each generation. The rats were bred at the same age as the first litter series and then rebred 1 week after the weaning of their first litters. In all cases the young rats were weaned at 21 days. In general, from each litter one male and two female rats which weighed the most at weaning were used for the growth experiments. The average age of the females when the second litters were born was between 171 and 190 days for the second to fifth generation and from 200 to 215 days in the generations following.

The experiments on stock rats were carried out during the winter of 1943–44 simultaneously with the ninth and tenth generations of the first litter rats, which received diet 57. All rats were originally from the same strain.

RESULTS

Figures 1 and 2 show the average weights of the various groups of male and female rats, respectively, at 21, 30, 60 and, in some cases, 90 and 120 days of age, as well as comparative values for stock rats maintained on our stock diet (diet 2)[3] as well as the values obtained by Sher-

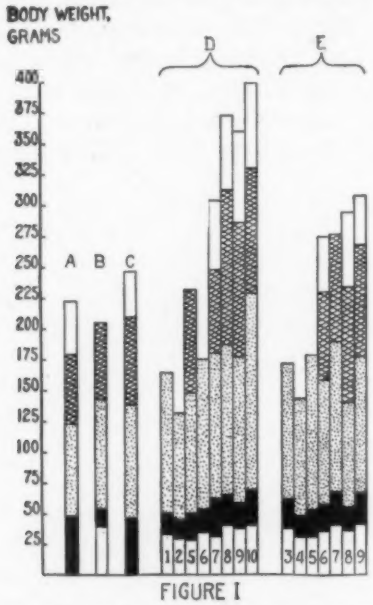

FIGURE I

Fig. 1 The body weight of male rats at 21 days (to top of lower blank space), at 30 days (to top of solid block), at 60 days (to top of stippled area), at 90 days (to top of cross-hatched area), and at 120 days (to top of upper blank space). The following letters designate the groups: A — Donaldson ('24); B — stock rats, University of Southern California; C — Sherman and Campbell ('24) on diet B; D — present tests with first litter rats; E — present tests with second litter rats. Figures in lower blank spaces in D and E indicate successive generations.

man and Campbell ('24) and the standard values of Donaldson ('24). The averages are in most cases for eight males and sixteen females in each group except in the stock rats for which they are the means of twenty-three males and twenty-three females which were average rats picked from twenty-three successive litters.[4]

[3] The stock diet (diet 2) had the following percentage composition: whole ground yellow corn, 45.0; whole wheat flour, 28.5; powdered skimmed milk, 18.0; alfalfa leaf meal, 4.0; brewer's yeast (Anheuser-Busch, Strain K), 2.0; cottonseed oil with vitamins A and D (6 parts cottonseed oil and 1 part superbiotol; mixture has 430 I.U. vitamin A and 60 I.U. vitamin D per pound), 1.5%; $CaCO_3$ (powdered), 0.5; and NaCl (commercial), 0.5.

[4] We wish to thank Miss Elizabeth G. Sumner who determined these values.

A summary of the data obtained from the breeding trials and pertinent results with respect to the litters are included in table 2.

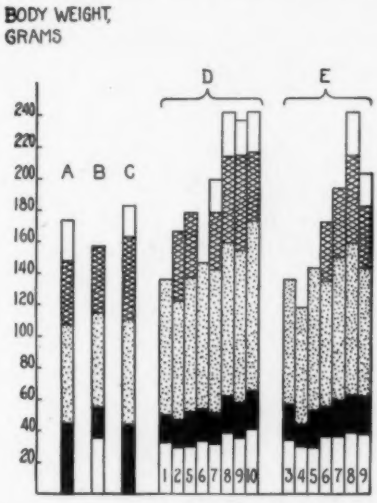

FIGURE II

Fig. 2 The body weight of female rats at 21 days (to top of lower blank space), at 30 days (to top of solid block), at 60 days (to top of stippled area), at 90 days (to top of cross-hatched area), and at 120 days (to top of upper blank space). The following letters designate the groups: A — Donaldson ('24); B — stock rats, University of Southern California; C — Sherman and Campbell ('24) on diet B; D — present tests with first litter rats; E — present tests with second litter rats. Figures in lower blank spaces in D and E indicate successive generations.

DISCUSSION

The results of the present experiments strongly support the view of Sherman and Campbell that diets satisfactory for growth and reproduction may not be optimal. Our stock diet (diet 2), which has been used successfully for 12 years with many generations in our stock colony, gives a rate of growth distinctly inferior to that obtained with the modified Sherman diet B (our diet 57) where the butterfat was replaced by a margarine fat enriched with vitamin A. The weight of male rats at 90 days of age on our stock diet averaged 205 gm. while the weights of the tenth generation of first litter rats on diet 57 had a mean average weight of 330 gm.; with the second litter rats on diet 57, the average value for the ninth generation was 267.5 gm. The growth also exceeds that obtained with rats of the Osborne and Mendel strain by Sherman and Campbell ('24) for males on their diet B (210.4 gm.) as well as the values of Donaldson ('24) in which the average weight is

TABLE 2

Summary of data obtained from breeding trials with successive generations of rats raised on diet 57 (a modified Sherman Diet B).

PARENTS					LITTER						
Generation	Females bred	Litters born	Average period after mating [1]	Average age of mother at birth of litter	Generation	Average number per litter	Average weight per rat		Number of rats weaned	Number of rats died	Number of litters destroyed [2] or stillborn
							At birth (*) or 3 days	At 21 days [2]			
First litter rats											
			days	*days*			*gm.*	*gm.*			
0	7	7	25.1	....	1	10.7	5.00*	31.1(35)	45	3	0
1	8	8	24.3	108.0	2	11.5	5.22*	29.3(42)	55	1	0
2	8	8	27.8	115.5	3	9.0	5.57*	.....	..	..	..
3	13	13	25.6	112.7	4	11.9	5.00*	28.4(77)	79	0	0
4	8	7	26.7	122.7	5	9.9	4.87*	27.6(35)	39	12	0
5	8	8	26.2	120.4	6	11.2	5.19*	32.4(56)	56	0	0
6	8	7	25.9	148.7	7	11.2	5.80	31.3(35)	38	4	1
7	8	6	26.0	149.0	8	9.8	8.02	39.3(35)	35	0	1
8	10	5	25.0	145.5	9	10.6	7.00	36.6(35)	35	0	0
9	14	12	28.0	149.8	10	8.4	6.88	39.2(42)	70	3	1
10	14	13	30.8	152.5	11	8.4	7.91	36.6(63)	75	0	2
Second litter rats											
2 [4]	8	8	27.8	176.5	3	7.8	5.99*	31.2(28)	39	7	0
3	8	8	24.4	171.5	4	12.1	5.35*	28.5(42)	58	0	0
4	8	8	25.3	191.3	5	10.4	4.99*	27.3(35)	47	14 [5]	0
5	8	5	31.8	185.2	6	10.4	6.76	31.6(7)	28	4	0
6	7	6	27.5	216.9	7	7.4	7.89	35.2(21)	29	3	1
7	8	7	25.0	197.5	8	8.7	7.83	34.1(35)	40	0	0
8	10	10	26.4	213.5	9	9.1	8.04	36.4(35)	60	8	0

[1] Period after male was placed in cage.

[2] Figures in parentheses are number of rats of which this value is the average. Only those litters where there were 7 in a litter at 21 days are included.

[3] Destroyed before 3 days.

[4] Second litter of first litter parents. The other rats in this group are from second litters of second litter parents.

[5] Includes 6 stillborn in one litter of 12 and 1 stillborn in a litter of 7.

184.8 gm. However, they fall far short of the maximum growth rate attainable in the Osborne and Mendel rats as reported by Anderson and Smith ('32). Similar variations in the weights of the female rats were observed, the 90-day averages being as follows: stock diet 2, 157 gm.; diet 57, tenth generation of first litter rats, 216 gm., and ninth generation of second litter rats, 182 gm. For comparison, the figures obtained by Sherman and Campbell for rats on diet B were 162.8 gm., and the values of Donaldson were 148 gm. However, because of differences in rate of growth not only of different strains of rats but also of different stock colonies of the same strain, it is not possible to make quantitative comparisons between different laboratories. Nevertheless, it would appear that our diet 57 is superior to our stock diet 2 to about the same extent that diet B excelled diet A in the Sherman-Campbell tests.

The number of females failing to give birth to young was 11% in the first litter group and 9% in the second litter rats. If one includes in this calculation the females whose litters were destroyed before 3 days, the figures become 16 and 11%, respectively, values comparing favorably with the 19% reported by Sherman and Campbell for their diet B in contrast to their value of 48% for diet A.

The results show a somewhat downward trend with respect to the weight at 21 days and the growth rate through the fourth generation and the marked improvement that followed, so that the results for the tenth generation of first litter rats and for the ninth generation of the second litter rats are the best of any group. The preliminary slump may have been due to the use of the commercial whole wheat flour which was later replaced by ground whole wheat prepared in our laboratory as needed.

It would also appear that the rate of growth is better in first litter rats than in the second litter although the 3-day and 21-day values are identical in the last litters reported in table 2. There also would seem to be a progressive improvement in weight of the first litter rats in the later generations. This may, in part, be traceable to selection of the more vigorous stock for breeding rather than as a result of diet. However, the growth studies in each generation were carried out on animals from all litters; in the later generations all of these were bred.

These results answer in the affirmative the question raised by Boutwell et al. ('43) as to the adequacy of a vegetable fat for continued growth and reproduction over a number of generations. Thus, a diet which contained no more butterfat than that present in skimmed milk powder to which was added as the main fat a vegetable margarine in

[6] Includes 6 stillborn in one litter of 12 and 1 stillborn in a litter of 7.

the proportion of fat found in whole milk powder, and which was supplemented once weekly with 5 gm. each of lean beef and lettuce, effectively supported growth and reproduction over ten generations.

SUMMARY

Experiments are reported on reproduction and growth rate of ten generations in which the lineage is through the first litter and of eight generations in which the lineage is through the second litters where the diets have been a modification of the Sherman diet B in which butterfat has been replaced by vitamin A-fortified margarine fat. The growth rate considerably exceeded that obtained with animals on our stock diet and progressively improved with the later generations. Somewhat faster growth was obtained with the first litter rats than with those in the second litter group. It is concluded that a vegetable fat such as that contained in a margarine can serve adequately in place of butterfat for growth and reproduction on a diet otherwise nutritionally satisfactory.

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COMPOSITION OF TYPICAL MEXICAN FOODS[1]

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A nutrition program cannot be properly planned in any country until data on the composition of its foods are at hand. On the basis of these data one may select foods which will integrate to form a nutritionally balanced menu at the lowest cost.

In preparing for a school lunch demonstration in Mexico City, it was first necessary to undertake the analysis of the major foods of Mexico because the few analyses that had been reported by Gavarre ('40), Gonzalez ('42), Alvarez ('42) and Zozaya and Alvaredo ('43) were incomplete. The compilation of data by Axtmayer and Cook ('42) indicates that little is known of the composition, especially the vitamin content, of Latin American foods.

In the present study 112 samples of food from Mexico were analyzed for carotene, thiamine, riboflavin, niacin, ascorbic acid, calcium, phosphorus, iron, nitrogen, ash and total solids content. To our knowledge many of these foods were analyzed for the first time. The seed and legume samples were harvested in 1942 and 1943, while most of the remaining samples were grown during the first half of 1944.

EXPERIMENTAL

1. Method of sampling and shipping

The fruit and vegetable samples were purchased in the public markets, usually in Mexico City, packed in waxed cartons, imbedded in carbon

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dioxide ice and while frozen shipped by air express to Boston. Preliminary tests by Lockhart et al. ('44) had shown no measurable vitamin losses during 4 days' storage under these conditions. All samples arrived within 3, and usually 2 days and were held in a carbon dioxide ice refrigerator until analyzed. The seed and legume samples were shipped by parcel post in cardboard or wooden cartons, and were stored in glass jars at room temperature until analyzed.

A portion of each sample was boiled in 3% metaphosphoric acid, macerated in a Waring Blendor, and analyzed immediately for ascorbic acid content. The remainder of each sample was macerated without pre-treatment, returned to the carton, and refrozen. Portions of this frozen sample were taken as required, but all vitamin analyses on a sample were made during the same day.

2. Methods of analysis

The A.O.A.C. ('40) spectrophotometric method was used to determine the carotene content of all samples except the dry chilis, for which the chromatographic procedure of Moore ('40) was used. Thiamine was measured according to Moyer and Tressler ('42), riboflavin according to Andrews ('43), niacin according to the U. S. Pharmacopeia ('43) and ascorbic acid by the Hochberg, Melnick and Oser ('43) modification of the method by Bessey and King ('33). Moisture, ash, nitrogen, calcium and manganese content were measured according to the A.O.A.C. ('40), phosphorus according to Fiske and Subba-Row ('25) and iron according to Koenig and Johnson ('43).

The results of these analyses are presented in tables 1 to 4. The foods have been arranged alphabetically according to the Spanish name. The English translation is given whenever it is known. These data are presented on the "wet basis", in terms of the foods as received, because they should be more useful in this form. The composition on the "dry basis" may easily be calculated.

DISCUSSION

The analyses of some of these foods do not always agree with previous reports in the scientific literature. Though the samples were carefully taken, and all shipments were received in excellent condition, the food may have been improperly handled previous to collection. Also, the difficulties inherent in sampling ½ pound amounts of a vegetable or fruit are great. That several samples must be obtained from several areas before one can be satisfied with the data on each food is

indicated by the results on six separate samples of malva (table 1). The considerable variation in the content of certain nutrients indicates that differences in botanical strain, in soil and in climate may affect the nutrient value of foods.

Food data are no more accurate than the methods of analysis used to obtain them. While the most acceptable chemical and physical methods were used in this investigation, there was some inaccuracy due to interference by chemical substances present in plant tissue. This interference may be especially misleading when a species of plant is analyzed for the first time.

1. Protein

No attempt has been made to calculate the protein content from the nitrogen values because the correct conversion factor is not yet known for many of these foods.

If the usual factor ($N \times 6.25$) were to be employed, at least ten of the foods contained over 22% protein. The foods richest in nitrogen content (expressed as gm./100 gm.) were: charales 9.9, parota 5.9, India squash seed 5.2, Castilla squash seed 5.0, peanut 4.4, guaje seed 4.2, sesame seeds 3.9, piñón 3.6, lentil 3.6, lima bean 3.6, palacio bean 3.6 and bayo gordo bean 3.6. Undoubtedly, the quality of the proteins of these foods differs. However, most are legumes, and legume proteins are generally superior to cereal proteins.

2. Calcium

A number of these foods were rich in calcium content, the most important (in mg./100 gm.) were: charales 4160, sesame seed 417, guaje seed 322, epazote 260, malva 257, bayo gordo bean 204, chipotle chile 195, leek 190, tuna roja 181, cocona bean 180, black bean 160, palacio bean 159, small alubia bean 157, hediondilla 155 and morita chili 153. The calcium in these foods may not be equally available. Charales were high in calcium primarily because of the skeletal tissue content. The unusually high quantity of calcium in malva deserves attention for, unlike many foods in this group, it is relatively easy for one to consume 100 gm. as a daily portion.

3. Phosphorus

The fourteen foods richest in phosphorus content (in mg./100 gm.) were: charales 2640, Castilla squash seed 834, India squash seed 655, piñón 588, sesame seed 566, parota 544, lima bean 439, guaje seed 411,

TABLE 1

Composition of vegetables.

ENGLISH NAME	SPANISH NAME	BOTANICAL NAME	CULTIVATED IN	CONTENTS PER 100 GM. AS RECEIVED										
				Water	Nitrogen	Ash	Calcium	Phosphorus	Iron	Carotene	Thiamine	Riboflavin	Niacin	Ascorbic acid
				gm.	gm.	gm.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
Chard	Acelga	Beta vulgaris, var. cycla	D. F.	89.3	0.52	2.4	71	36	2.5	4.35	0.06	0.21	0.68	8.0
Watercress	Berro	Nasturtium aquaticum L.	Morelos	93.3	0.39	1.3	122	59	2.4	1.04	0.07	0.13	1.04	3.2
Beets	Betabel	Beta vulgaris, var. rapacea	D. F.	82.3	0.53	1.0	20	73	1.3	0.00	0.05	0.05	0.38	29.4
Squash, small	Calabacitas	Cucurbita mexicana L.	Morelos	92.6	0.29	0.7	14	53	16.4	0.29	0.08	0.08	0.56	8.8
Squash blossoms	Calabacitas, flor de	Cucurbita mexicana L.	D. F.	92.2	0.28	1.2	29	167	1.3	0.94	0.16	0.20	0.98	3.9
Potato, sweet	Camote	Ipomoca batatas L.	Morelos	60.7	0.10	0.8	39	27	1.2	0.45	0.09	0.03	0.28	21.3
.....	Chayote con espinas	Sechium edule, Swartz	Morelos	92.1	0.13	0.4	7	26	0.4	0.03	0.03	0.02	0.22	25.2
.....	Chayote sin espinas	Sechium edule, Swartz	Veracruz	88.6	0.16	0.6	27	34	1.0	0.00	0.03	0.07	0.43	7.6
Peas, green	Chicharos	Pisum sativum, L.	México	75.6	1.28	0.7	27	97	2.3	0.57	0.33	0.10	2.33	67.0
.....	Chinchayote	Sechium edule Sw.		74.6	0.33	1.0	13	68	1.7	0.01	0.01	0.04	1.53	10.4
(Root of chayote)	Chilacayote	Cucurbita filicifolia Bouch		94.2	0.20	0.5	22	28	0.6	0.06	0.03	0.08	0.38	1.9
Coriander	Cilantro	Coriandrum sativum, L.	D. F.	88.6	0.51	1.6	110	45	2.0	4.31	0.13	0.02	0.99	10.6
Cabbage	Col	Brassica oleracea, L. var. capitata	D. F.	89.6	0.37	0.6	29	42	1.1	0.14	0.11	0.06	0.40	20.4
Cauliflower	Coliflor	Brassica oleracea, L. var. botrytis	D. F.	90.0	0.45	1.0	33	66	3.7	0.05	0.15	0.10	0.69	153.7
Beans, green	Ejote	Phaseolus vulgaris	Morelos	88.7	3.41	0.7	51	49	2.5	0.42	...	0.09	0.50	11.5
Corn, fresh white	Elotes, blanco	Zea mays		65.7	0.45	0.7	32	44	1.2	0.00	0.16	0.09	1.52	99.7
.....	Endivia	Sonchus oleraceus	Hidalgo	92.0	0.39	1.7	93	35	12.5	4.99	0.07	0.12	0.43	4.7
Chenopodium	Epazote	Chenopodium ambrosioides	Morelos	89.6	0.46	2.2	260	39	5.4	1.02	0.04	0.13	0.57	3.7
Spinach	Espinaca	Spinacia oleracea, L.	D. F.	88.4	0.57	2.3	74	37	7.5	4.43	0.20	0.25	0.65	82.1
				83.3	0.81	1.7	150	46	4.3	2.36	0.09	0.36	1.10	8.9
.....	Jicama	Pachirhizus palmatilobus	Morelos	91.8	0.07	0.3	11	13	0.5	0.03	0.02	0.01	0.11	13.7

Chenopodium	Epazote												
Spinach	Espinaca	Spinacia oleracea, L.	D. F.	88.4	0.57	2.2	74	37	7.5	4.43	0.20	0.25	0.65	82.1
				83.2	0.81	1.7	150	46	4.3	2.26	0.09	0.36	1.10	8.9
.....	Jicama	Pachirhizus palmatilobus	Morelos	91.8	0.07	0.3	11	13	0.5	0.03	0.02	0.01	0.11	13.7
Lettuce	Lechuga	Lactuca sativa, L.	D. F.	96.6	0.23	0.6	31	30	1.2	0.61	0.11	0.06	0.25	3.0
.....	Lengua de vaca	Rumex crispus L.	D. F.	93.0	0.52	1.8	82	69	6.4	4.10	0.09	0.13	0.20	...
				93.0[1]	0.25	1.2	93	29	5.2	1.33	0.07	0.19	0.61	31.7
Century plant flower	Maguey, flor de	Agave atrovirene	Hidalgo	91.0	0.15	0.6	114	27	0.9	0.43	0.11	0.05	0.21	58.6
Malva	Malva	Malva parviflora	Hidalgo	85.9	0.94	2.6	312	73	19.5	2.53	0.11	0.19	0.95	45.4
		Malva parviflora	D. F.	92.4	0.34	2.2	268	42	9.2	2.40	0.07	0.17	0.51	...
		Malva vastrum peruvianum	D. F.	91.2	0.40	2.2	292	29	8.7	4.31	0.07	0.22	0.45	...
		Malva silvestris	D. F.	92.0	0.33	1.7	221	36	9.7	4.80	0.10	0.24	0.44	...
		Malva parviflora	D. F.	82.9	0.96	2.9	188	111	11.3	8.50	0.16	0.26	1.24	14.6
		Modiola multifida M.	D. F.	91.5	0.35	3.6	263	39	6.0	2.13	0.08	0.16	0.38	...
.....	Mezquite	Prosopis juliflora L.	Hidalgo	71.5	1.02	1.0	140	57	0.1	0.15	0.05	0.05	0.95	...
Turnip, flower	Nabo, flor de	Brassica campestris	D. F.	87.4	0.67	1.5	117	97	3.4	3.66	0.04	0.02	1.21	41.6
Turnip, leaves	Nabo, hojas de	Brassica campestris	D. F.	84.0	0.58	1.0	138	38	5.2	2.84	0.06	0.13	0.52	147.3
Cactus leaf	Nopales	Opuntia hyptiacantha	Mexico	92.5	0.21	1.0	105	27	0.8	0.50	0.04	0.04	0.30	4.1
Cucumber	Pepino	Cucumis sativus	D. F.	95.4	0.12	0.5	18	32	0.5	0.11	0.03	0.04	0.18	17.1
Parsley	Perejil	Carum petroselium Benth.	D. F.	84.8	0.65	1.9	152	55	4.1	4.56	0.16	0.37	1.95	12.1
Leek	Porro	Allium porrum L.	D. F.	71.5	0.20	0.7	40	46	1.0	0.05	0.10	0.06	0.39	6.3
.....	Quelites	Amaranthus chlorostachys	Morelos	86.1	0.64	3.0	190	39	4.6	4.60	0.12	0.19	0.57	67.9
.....	Romerito	Dondia sufrutencona	D. F.	92.0	0.58	1.8	41	18	2.5	2.76	0.12	0.08	0.30	4.4
.....	Tejocotes	Crataegus mexicana Moc. et Sess	D. F.	76.1	0.15	0.7	91	37	1.2	6.40	0.05	0.05	0.48	18.6
Purslane	Verdolagas	Portulaca oleracea, L.	Morelos	90.7	0.36	1.9	86	40	5.2	3.25	0.08	0.11	0.56	10.8
.....	Xocoyol	Oxalis divergens	Hidalgo	91.0	0.68	0.9	79	38	5.1	3.70	0.12	0.12	0.42	26.3
				92.6	0.67	0.8	182	32	3.5	3.34	0.11	0.15	0.48	...
Yucca flower	Yuca, flor de	Yucca alcifolia, L.	Hidalgo	90.0	0.49	0.7	47	73	0.5	0.02	0.14	0.09	0.58	11.7
Carrot	Zanahoria	Daucus carota L.	D. F.	91.1	0.08	0.6	20	30	0.0	5.14	0.05	0.03	0.30	26.8

[1] Ground with moisture added. Sample used for all analyses except ascorbic acid.

TABLE 2 *

Composition of fruits.

ENGLISH NAME	SPANISH NAME	BOTANICAL NAME	CULTIVATED IN	CONTENTS PER 100 GM. AS RECEIVED										
				Water	Nitrogen	Ash	Calcium	Phosphorus	Iron	Carotene	Thiamine	Riboflavin	Niacin	Ascorbic acid
				gm.	gm.	gm.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
Avocado	Aguacate	Persea gratissima L.	Morelos	79.7	...	0.8	16	47	1.03	0.18	0.07	0.10	0.80	33.3
Wild cherry	Capulín	Prunus capuli L.	D. F.	81.2	0.18	0.6	18	24	0.72	0.51	0.03	0.03	0.65	8.4
Apricots	Chavacono	Prunus armeniaca, L.	Puebla	83.4	0.09	0.8	17	32	1.20	2.54	0.03	0.03	0.41	2.5
Custard apple	Chirimoya	Annona cherimolla	D. F.	30.6	0.42	0.8	23	43	0.41	0.02	0.13	0.15	2.03	6.8
Plums	Ciruelas	Spondias purpurpea L.	Guerrero	86.5	...	0.4	44	24	0.94	0.38	0.05	0.02	0.26	45.5
Strawberry	Fresa	Fragaria mexicana	Gto.	91.0	0.13	0.5	28	30	2.12	0.06	0.03	0.03	0.28	27.8
.....	Granada China	Passiflora ligularis	Puebla	78.5	0.44	1.4	53	71	1.27	0.25	0.01	0.10	1.51	2.0
Guavas	Guayaba	Psidium guayava L.	Morelos	81.5	0.15	0.7	30	29	0.70	1.47	0.06	0.04	1.20	89.4
Figs	Higo	Ficus carica	D. F.	83.0	0.21	0.8	65	22	0.38	0.13	0.05	0.05	0.44	3.6
Tomato	Jitomate	Lycopersicum esculentum	D. F.	94.6	0.12	0.5	6	22	0.36	3.54	0.06	0.03	0.37	17.9
.....	Lima	Citrus limetta	Guerrero	91.7	0.09	0.3	16	15	0.21	0.02	0.04	0.02	0.14	51.0
.....	Limón agrio	Citrus limonia O.	Veracruz	88.4	0.15	0.5	5	27	0.66	0.04	0.05	0.03	0.20	18.6
.....	Limón real	Citrus médica	Puebla	90.3	0.09	0.4	25	24	0.21	0.04	0.12	0.02	0.33	28.1
.....	Mamey	Calocarpum mamosum	Puebla	71.4	0.18	1.6	36	38	0.37	1.46	0.03	0.00	2.14	22.7
Tangerine	Mandarina	Citrus nobilis var. deliciosa Swingle	San Luis Potosi	84.8	0.16	0.4	69	22	0.37	1.81	0.10	0.03	0.22	111.8
Manila mango	Mango (Manila)	Mangifera indica var. manila	Oaxaca	79.6	0.12	0.4	7	16	0.23	1.96	0.14	0.05	1.64	171.1
Common mango	Mango (Corriente)	Mangifera indica	Oaxaca	83.2	0.11	0.4	19	6	0.30	1.17	0.01	0.02	0.21	33.3
Muskmelon	Melón	Cucumis melo L.	Morelos	93.5	0.10	0.5	15	11	0.79	1.68	0.03	0.02	0.53	23.6
Orange	Naranja	Citrus aurantium	Veracruz	86.6	0.13	0.4	33	17	0.20	0.29	0.09	0.03	0.25	182.3
Papaya	Papaya	Carica papaya L.	Veracruz	91.2	0.09	0.6	36	23	0.37	1.41	0.02	0.03	0.28	64.8
.....	Perón	Pyrus malus	Puebla	83.4	0.04	0.5	9	15	0.66	0.02	0.04	0.02	0.15	...
Pineapple	Piña	Ananas nativum	Veracruz	88.5	0.08	0.3	13	5	0.41	0.18	0.11	0.03	0.18	41.1
Banana	Platano macho	Musa paradesiaca	Veracruz	78.6	...	0.8	10	34	0.55	0.28	0.03	0.05	0.65	10.6
Banana	Platano tabasco	Musa sapientum	Chiapas	60.2	...	0.8	7	40	0.93	1.95	0.05	0.02	0.52	17.5
.....	Tomate	Physalis aequata	Morelos	92.5	0.22	0.4	9	33	0.70	0.19	0.07	0.03	0.66	76.4
.....	Tuna, blanca	Opuntia hyptiacantha	Hidalgo	86.2	...	0.4	36	34	0.50	0.08	0.01	0.02	...	...
.....	Tuna, colorada	Opuntia robusta	Hidalgo	82.0	...	0.7	63	32	0.70	0.12	0.01	0.02	...	...
.....	.....		Hidalgo	89.3	0.10	1.3	181	11	0.93	0.01	0.01	0.02	0.24	19.1
.....	Xoconoxtle	Opuntia imbricata	Hidalgo	83.5	...	0.7	110	14	0.50	0.05	0.45	0.23	0.15	21.9
Sapote, white	Zapote, blanco	Casimiroa edulis	Puebla	89.3	0.25	0.4	8	19	0.23	0.03	0.03	0.06	1.00	15.7
Sapote, small	Zapote, chico	Achras zapota L.	Veracruz	78.5	0.09	0.5	22	6	0.63	0.05	0.02	...	0.24	6.3
Sapote, black	Zapote, negro	Diospiros ebenaster	Morelos	83.1	0.07	0.6	22	23	0.26	0.19	0.00	0.02	0.20	191.7

TABLE 3

Composition of seeds and legumes.

Sapote, small	Zapote, chico	Achras zapota L.	Veracruz	79.5	0.59	0.5	22	6	0.03	0.03	0.02	0.24	0.5	
Sapote, black	Zapote, negro	Diospiros ebenaster	Morelos	83.1	0.07	0.6	22	23	0.26	0.19	0.00	0.02	0.20	191.7

TABLE 2

Composition of seeds and legumes.

ENGLISH NAME	SPANISH NAME	BOTANICAL NAME	CULTIVATED IN	CONTENTS PER 100 GM. AS RECEIVED										
				Water	Nitrogen	Ash	Calcium	Phosphorus	Iron	Carotene	Thiamine	Riboflavin	Niacin	Ascorbic acid
				gm.	*gm.*	*gm.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Sesame seed	Ajonjolí	Sesamum indicum L.	Guerrero	3.8	3.88	4.4	417	566	8.4	0.02	1.36	0.23	5.01	0.5
Bean	Alubia Chica	Phaseolus vulgaris L.	Querétaro	9.2	3.23	3.7	157	406	6.6	0.01	0.11	0.18	1.82	3.1
Bean	Alubia grande	Phaseolus vulgaris L.	Querétaro	11.4	3.21	3.7	108	362	6.6	0.05	0.81	0.20	2.14	3.0
Rice	Arroz	Oriza sativa L.	Morelos	12.0	1.40	0.8	8	172	0.8	0.00	0.32	0.03	2.40	0.0
Peas (dried)	Alverjon	Pisum sativum	Puebla	10.2	3.27	3.1	72	287	7.5	0.02	0.91	0.18	2.30	0.0
Oats	Avena	Avena sativa L.	México	6.3	2.58	1.5	61	278	3.3	0.00	0.53	0.11	0.82	0.0
Peanut	Cacahuate	Arachis hipogea L.	Guanajuato	5.1	4.40	2.0	49	292	2.1	0.01	0.95	0.13	14.42	0.7
Sugar cane	Caña	Saccharum officinarum var. F. C916	Morelos	74.4	0.04	0.3	21	10	0.6	0.00	0.36	0.01	0.10	0.0
Beans, ayocote	Frijol ayocote	Phaseolus vulgaris L.	Guanajuato	11.7	2.39	3.6	116	262	5.9	0.03	0.42	0.19	1.92	1.3
Bean, bayo gordo	Frijol bayo gordo	Phaseolus vulgaris L.	Zacatecas	10.1	3.56	3.6	204	291	5.5	0.04	0.75	0.22	1.19	0.9
Bean, cocona	Frijol cocona	Phaseolus vulgaris L.	Puebla	10.9	3.27	3.6	155	382	11.5	0.00	0.82	0.15	1.37	1.4
			Guanajuato	9.5	3.28	3.8	206	320	7.1	0.02	1.14	0.17	1.64	3.3
Bean, black	Frijol negro	Phaseolus vulgaris L.	Puebla	13.7	3.11	3.4	142	382	8.7	0.04	0.81	0.22	1.69	1.1
			Querétaro	9.5	3.26	3.6	177	345	7.3	0.01	0.96	0.18	1.86	4.4
Bean, palacio	Frijol palacio	Phaseolus vulgaris L.	Guanajuato	10.6	3.55	3.3	159	369	6.9	0.03	0.85	0.13	1.57	2.1
Pea, chick (dried)	Garbanzo breve	Cicer arientium L.	Sonora	9.1	3.44	3.4	100	403	9.3	0.08	0.84	0.18	1.67	1.3
			Sonora	7.7	3.27	3.4	110	399	8.4	0.05	0.65	0.16	1.41	0.0
Bean, lima	Haba	Faba vulgaris, M vicia faba L.	Puebla	8.9	3.63	3.2	49	439	7.3	0.08	0.91	0.31	2.30	4.9
Lentil	Lenteja	Ervum lens L.	Querétaro	11.2	3.62	2.5	82	307	5.4	0.09	0.73	0.21	1.96	7.5
			Querétaro	9.4	3.56	3.0	86	404	5.8	0.90	0.67	0.16	1.51	3.2
Corn, white	Maíz	Zea mays L.		12.2	1.29	1.2	7	253	2.5	0.03	0.32	0.08	1.58	1.3
			Edo. Mexico	10.5	1.19	1.3	10	261	2.8	0.03	0.42	0.09	1.70	1.9
.....	Parota	Enterolobium ciclocarpum	Morelos	1.4	5.88	3.2	29	544	4.2	0.04	2.72	0.21	1.75	6.6
.....	Piñón	Pinus edulis Eng. P. cembroides		2.3	3.59	2.9	13	588	5.2	0.02	1.11	0.24	5.29	0.9
Seed of Castilla squash	Semilla de Calabaza de Castilla	Cucurbita pepo L.	Morelos	4.9	4.96	5.1	39	834	11.0	0.05	0.26	0.17	2.30	0.0
Seed of India squash	Semilla de Calabaza de India	Cucurbita pepo L.	Morelos	4.2	5.17	4.9	37	655	8.9	0.06	0.36	0.23	3.28	0.1
.....	Semilla de Guaje	Leucanea suculenta L.	Morelos	7.8	4.22	5.0	322	411	15.2	2.25	1.44	0.21	4.00	31.3
Wheat	Trigo	Triticum vulgare L.	Guanajuato	10.1	1.56	1.7	50	290	9.2	0.01	0.44	0.10	3.68	0.0

TABLE 4

Composition of chilis and chili seed (Semilla) and miscellaneous foods.

ENGLISH NAME	SPANISH NAME	BOTANICAL NAME	CULTIVATED IN	CONTENTS PER 100 GM. AS RECEIVED										
				Water	Nitrogen	Ash	Calcium	Phosphorus	Iron	Carotene	Thiamine	Riboflavin	Niacin	Ascorbic acid
				gm.	*gm.*	*gm.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Chili, dry	Chile seco													
	Ancho	Capsicum annum L. grossum Sendt	Aguascalientes	10.0	1.57	6.0	70	212	5.7	38.94	0.16	0.71	3.44	143.0
	Semilla			6.5	2.97	3.7	32	446	7.3	0.20	1.28	0.15	8.20	64.8
	Cascabel	Capsicum annum L. cerasiforma Mill.	Jalisco	9.5	1.67	7.5	75	216	4.7	18.60	0.14	0.54	9.70	58.8
	Semilla			7.4	2.42	2.9	42	472	5.3	0.40	0.91	0.18	10.20	44.1
	Chipotle	Capsicum annum L. dulce Hort	Hidalgo	16.1	1.95	6.6	195	281	6.1	6.05	0.33	0.62	13.00	24.2
	Semilla			8.3	2.94	3.8	68	517	6.2	0.26	0.93	0.35	9.80	40.5
	Guajillo	Capsicum annum L. longum Sendt	Aguascalientes	17.6	1.68	7.2	91	189	10.1	44.40	0.16	0.76	4.50	135.0
	Semilla			5.6	2.60	3.3	44	476	7.1	0.45	1.92	0.21	11.10	34.4
	Morita	Capsicum annum L. abreviatum Fing	Puebla	14.0	2.28	7.1	153	157	5.2	10.50	0.23	1.37	15.40	98.6
	Semilla			9.2	2.87	3.7	79	457	5.6	0.51	2.23	0.50	11.29	209.0
	Mulato	Capsicum annum L. grossum Sendt	Puebla	15.3	1.43	5.0	80	199	12.8	39.00	0.29	0.60	4.09	111.5
	Semilla			6.8	2.64	3.4	45	492	8.3	0.40	2.64	0.19	7.80	36.4
	Pasilla	Capsicum annum L. longum Sendt	Jalisco	15.9	1.72	6.2	105	163	6.3	56.60	0.43	1.00	7.84	54.3
	Semilla			5.8	2.63	3.0	37	422	7.4	0.12	1.28	0.23	9.50	56.3
	Piquin con semillas	Capsicum frutescens L. var. baccalum	Hidalgo	9.5	2.27	4.0	127	320	7.8	4.50	0.56	0.44	15.20	71.1
Chili, fresh	Chile verde													
	Jalapeño	Capsicum annum L.	Veracruz	92.1	0.22	0.5	12	29	0.5	0.33	0.05	0.03	0.65	75.2
	Mulato	Capsicum annum L. grossum Sendt	Tamaulipas	89.2	0.32	0.6	17	35	1.4	1.18	0.17	0.06	1.10	174.5
	Serrano	Capsicum annum L. acuminatum Fing	Veracruz	86.8	0.43	0.8	25	54	0.8	0.55	0.11	0.06	1.52	57.9
Miscellaneous	Agua miel		Hidalgo	87.8	...	..	10	20	0.4	0.00	0.10	0.01	0.50	11.3
	Charales		D. F.	3.9	9.9	..	4160	2640	22.7	3200[1]	0.40	0.10	6.00	0.0
	Gusanos	Hipopta agavis	Hidalgo	66.9	1.8	..	...	...	...	0.05	0.49	0.37	2.40	3.9
	Pulque		D. F.	97.0	0.7	..	10	10	0.7	0.00	0.20	0.02	0.30	6.2
	Quesa de tuna		D. F.	17.2	0.2	..	51	29	13.0	0.10	0.20	0.20	1.50	88.0
	Tortilla			38.9	0.8	1.0	131	195	2.8	0.19	0.18	0.05	0.89	0.0
				43.2	1.0	0.9	101	178	1.9	...	0.21	0.07	1.07	..
				43.7	0.9	0.9	101	178	1.8	...	0.19	0.06	0.93	..

[1] I.U. of vitamin A.

small alubia bean 406, chick pea 401, palacio bean 369, black bean 363, large alubia bean 362 and cocona bean 351.

4. Calcium: phosphorus ratio

Nutritionally, foods rich in phosphorus content may be undesirable, especially for populations like the Mexican, whose intake of calcium and vitamin D may be limited. It is generally agreed that a calcium: phosphorus ratio of approximately 1:1 is best in the human dietary. The calcium: phosphorus ratios of those foods rich in either of these minerals were as follows: epazote 6.7, malva 4.7, charales 1.58, morita chili 0.97, guaje seed 0.78, sesame seed 0.74, bayo gordo bean 0.70, chipotle chili 0.69, cocona beans 0.46, black bean 0.44, palacio bean 0.43, small alubia bean 0.39. The Ca:P ratio of large alubia beans, chick pea, lima bean, India squash seed, Castilla squash seed, parota and piñón were each 0.30, or below. Thus it is evident that epazote, malva and charales are foods which may be especially useful for calcium and phosphorus metabolism.

5. Iron

The foods highest in iron content (in mg./100 gm.) were: charales 23, calabacitas 16, guaje seed 15, quesa de tuna 13, mulato chili 13, endivia 13, cocona bean 12, malva 11, Castilla squash seed 11, hediondilla 9, India squash seed 9, wheat 9 and chick peas 9. There are few foods in the American dietary as rich as these in iron content. The nutritional availability of the iron in Mexican foods has not yet been determined.

6. Carotene

The carotene content of the samples of dry chili ranged between 5 and 57 mg. (equivalent to 8200 and 95000 I.U. of vitamin A) per 100 gm. Only 6 gm. of pasilla chili would be needed to supply the entire daily allowance advised by the Food and Nutrition Board ('43) for an adult man. It is evident that chili can be an important source of provitamin A in the Mexican dietary. Other foods rich in carotene (expressed as mg./100 gm.) were: tejocote 6, carrot 5, endivia 5, quelites 5, parsley 5, lengua de vaca 5, chard 4, cilantro 4, spinach 4, malva 4, coriander 4, turnip flower 4 and jitomate 4. All of these contain at least 7000 I.U. of vitamin A per 100 gm., assuming a direct conversion factor for carotene.

7. Thiamine

The foods richest in thiamine content (in mg./100 gm.) were: parota 2.7, chili seed 1.3 to 2.6, guaje seed 1.4, sesame seed 1.4, piñón 1.1, pea-

nut 1.0, cocona bean 1.0, lima bean 0.9 and alverjon 0.9. The high content of thiamine in parota is notable.

8. Riboflavin

None of these foods was rich in riboflavin content. Dry chili contained 0.4 to 1.4 mg. per 100 gm. but a day's portion of chili can hardly be considered a significant source. The other foods highest in this vitamin (in mg./100 gm.) were: gusanos 0.4, parsley 0.4, lima bean 0.3, spinach 0.3, hediondilla 0.3, piñón 0.2, India squash seed 0.2, xoconoxtle 0.2, sesame seed 0.2, bayo gordo bean 0.2, black bean 0.2, quesa de tuna 0.2, guaje seed 0.2, alubia bean 0.2, malva 0.2, and chard 0.2.

9. Niacin

Though some chilis contained much niacin, peanuts were the most important source of this vitamin. The foods richest in niacin (as mg./100 gm.) were: dry chili 3.5 to 15.4, peanuts 14.4, charales 6.0, piñón 5.3, sesame seed 5.0, guaje seed 4.0, wheat 3.7, India squash seed 3.3, gusanos 2.4, rice 2.4, Castilla squash seed 2.3, green peas 2.3, alverjon 2.3, lima bean 2.3, mamey 2.1, large alubia 2.1 and custard apple 2.0.

10. Ascorbic acid

Several foods were exceptionally rich in this vitamin. The contents (mg./100 gm.) found were: black sapote 192, orange 182, mulato chili (green) 175, manila mango 171, cauliflower 154, turnip leaves 147, ancho chili 143, guajillo chili 135, tangerine 112, mulato chili 111, guava 89, quesa de tuna 88, spinach 82, tomato 76, quelites 68, green peas 67, papaya 65, maguey flower 59, lima 51, plums 46 and malva 45.

11. Manganese

During the investigation, it was noticed that the ash of cilantro was unusually green in color; later it was proven to be due to an extremely high manganese content (12.9 mg. %). In Sherman's tables ('41), the American food reported with the highest manganese content is oatmeal with only 4.9 mg. per 100 gm.

12. General considerations

The value of a food in supplying the needs of a population group is measured by the nutrient content in a daily serving rather than the content per 100 gm. Thus, although chili is rich in ascorbic acid, a large

amount cannot be eaten daily. On the other hand, cereals which are relatively low in nutrient content become important sources because of the large quantities consumed.

Some exceptional foods have been revealed by these analyses. Malva is an uncultivated plant that grows abundantly on the Mexican plateau. It is cooked in much the same manner as spinach which it resembles in taste, though malva is more fibrous. It is interesting that an ordinary portion (100 gm.) of malva contains approximately 40% of the calcium, 90% of the iron, 140% of the vitamin A (as carotene) and 60% of the ascorbic acid allowances for an adult man proposed by the National Research Council. The variations of the analyses of the several samples of malva indicate that more nutritive strains can be expected through breeding and cultivation.

Charal is an inexpensive air-dried fresh water fish, usually 3 cm. to 6 cm. in length, the entire carcass of which is customarily eaten. Charales are especially rich in protein and calcium for a 30-gm. portion supplies approximately 27% of the protein and 155% of the calcium allowances of adult man.

Quesa de tuna is prepared from the fruit of the prickly pear cactus and resembles cheese in texture. Its nutritive value lies especially in its iron and ascorbic acid content. Gusanos are grubs gathered from the roots of the maguey cactus and served as a fried delicacy. They are low in nutritive value.

Pulque is a beverage prepared by the fermentation of the juice of the maguey cactus. In arid areas of the Mexican plateau, it is consumed in liberal amounts, partly to supply water. It is an inexpensive sour drink which in daily portions of 500 ml. furnishes significant quantities of minerals and vitamins, especially ascorbic acid. The ascorbic acid analysis has been confirmed by bioassay using guinea pigs.

The results indicate that sesame seed is a food rich in calcium, iron, thiamine, and niacin; guaje seed is rich in calcium, iron, carotene and thiamine; charales in protein, calcium, iron, vitamin A and niacin; parota in protein, thiamine and niacin; peanuts in protein and niacin; and calabaza seed in protein, iron and niacin. In the quantities consumed, pulque is a good source of thiamine and ascorbic acid. Piñón contains an abundance of protein and niacin, and many of the beans are rich in protein, iron, calcium and niacin.

The exceptional amounts of calcium, iron, carotene, thiamine and protein found in these Mexican foods suggest that it may be possible to nourish the Mexican people without the use of dairy and meat products. These results indicate that the food pattern of Mexico is

quite different from that of the United States. Thus it would be inadvisable to base the Mexican nutrition program upon that of the United States. Instead, this program should be developed upon the nutrient composition of Mexican foods. Finally, the data of this analysis of more than 100 foods indicate that the Mexican dietary may be more adequate in ascorbic acid, phosphorus, calcium and thiamine than in riboflavin, niacin and quality-protein.

CONCLUSIONS

1. The results of the analysis of 112 samples of Mexican foods for carotene, thiamine, riboflavin, niacin, ascorbic acid, calcium, phosphorus, iron, nitrogen, ash and total solids content are presented.
2. Malva, sesame seed, charales, epazote, parota, peanut, guaje seed and calabaza seed were found to be especially nutritious foods. Many Mexican foods are richer in specific nutrients than foods in the United States.
3. The kinds and nutritional qualities of the foods of Mexico are so different from those of the United States that the nutrition program in Mexico should be based upon the nutrient content of Mexican foods.
4. It is probable that the Mexican diet is more adequate in ascorbic acid, thiamine, phosphorus, and calcium content than in riboflavin, niacin and quality-protein content.

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A STUDY OF NICOTINIC ACID RESTRICTION IN MAN[1]

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ONE FIGURE

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With the observation by Perlzweig, Levy and Sarett ('40) that the chief end product of nicotinic acid metabolism is to be found in the trigonelline fraction, and with the development of a direct method for the determination of trigonelline by Kodicek and Wang ('41), our interest was directed to the study of nicotinic acid metabolism in man with dietary restriction.

Several subjects volunteered to restrict their eating to a pellagra producing diet, low in trigonelline as well as nicotinic acid, with hopes of ridding themselves of some real or fancied ailment. Two of these adhered to the diet for a considerable period; it is with the results on these two subjects that the present report is particularly concerned.

METHODS AND MATERIAL

The subjects

Etta R. was a colored female, age 58, with complaints of stomach trouble and weakness. On a previous admission in 1930 she had pellagra. In 1936 she was operated on for removal of a uterine fibroid.

Examination revealed nothing of interest except possible cheilosis at one angle of the mouth and some pigmentation over the elbows. The tongue appeared somewhat atrophic but not red.

She adhered to the diet for 42 weeks. Throughout the greater part of the study she seemed to be contented; probably the diet and social life on the ward compared favorably with that at her home.

Chas. G. was a vagrant white male, a chronic alcoholic. He had been admitted several times previously with pellagra, and it had been customary with these admissions to put him on a pellagra producing diet for a variable period of study, so that he had come to look upon the diet as part of the cure.

[1] The expense of this study was supported in part by a grant-in-aid from the John and Mary R. Markle Foundation.

At the present admission he was mildly intoxicated and rather badly disoriented but he was able to indicate that he wanted the usual treatment. The skin over his neck, hands and wrists showed what might have been the result of sunburn ± pellagra — very red, rather rough but without vesicles. The tongue looked fairly good. Because of his mental state and the appearance of his skin and his past history, he was suspected of being in a state of nicotinic acid deficiency. He was immediately placed on the basal diet[2] made up of common foods. The nicotinic acid content of a duplicate of this diet was found to be 2.42 mg. by biological assay.

Supplementary foodstuffs were found to have nicotinic acid, milligram per 100 gm., as follows: squash 0.44 and 0.52; white potatoes 0.73; spaghetti 1.2; onions 0.19; cabbage 0.45. The following supplements were added to the diet of Etta R.: none during the first 18 weeks and none the last 2 weeks; 50 gm. cabbage and 50 gm. onions from the eighteenth through the thirtieth week; 50 gm. cabbage and 50 gm. squash during the thirty-first and thirty-second weeks; 50 gm. cabbage and 50 gm. white potatoes from the thirty-second to the fortieth week. A supplement of 50 gm. of spaghetti with 5 gm. of cheese was included in the diet of Chas. G. throughout his study. It was assumed that the 5 gm. cheese contained no significant quantities of nicotinic acid bodies.

Chemical technique

Twenty-four hour urines were collected in gallon bottles containing 25 ml. of 5 N HCl as preservative. Each week four of the daily specimens were analyzed for creatinine, trigonelline and nicotinic acid (nicotinic acid plus easily hydrolysable nicotinic acid derivatives). All determinations were made by colorimetric methods, employing the Evelyn instrument.

Creatinine was determined by an adaptation of the usual Folin and Wu method.

Nicotinic acid was determined by a technique involving the following steps: hydrolysis with N NaOH by immersion for 45 minutes in boiling water; acidification and adsorption on Lloyd's reagent; elution with 0.5 N NaOH; neutralization and clearing of the eluate with 10% $ZnSO_4$; and finally, color development with CNBr and Elon in the presence of acid phosphate buffer.

[2] The composition of the basal diet was: ground lean beef 25 gm., flour (not enriched) 120 gm., hominy 50 gm., oatmeal 25 gm., cream 20 gm., lard 25 gm., Nucoa 40 gm., simple syrup 150 gm., sugar as requested, synthetic fruit juice 3 servings, hard candy as requested.

Trigonelline was determined by the method of Kodicek and Wang ('41) with a few minor alterations.

Determinations were also made of the urinary output of the fluorescent substance F_2, by the method of Najjar ('44).

CLINICAL OBSERVATIONS

With a view towards increasing the nicotinic acid requirements, and relative deficiency, of the subject Etta R., 10 units of insulin were administered three times daily during the first 18 weeks. Following this she was given ultraviolet irradiation daily during the thirty-first to thirty-sixth weeks (cold quartz to entire body, 2 minutes each to anterior and posterior surfaces at 32-inch distance). Then she was exposed to x-rays during the thirty-ninth week, 200 R over the abdomen, alternating daily from anterior to posterior surfaces, and after 7 days of this two additional treatments of 150 R, the dosage being reduced because of nausea.

In spite of the restriction of nicotinic acid, irradiation, etc., nothing developed which could be called pellagra. There was some marginal redness of the tongue and some slight increase in the pigmentation over the elbows; we would have considered her condition, from the clinical standpoint, as mild nicotinic acid deficiency.

We were particularly surprised that so little effect appeared to result from the irradiation. In other patients irradiation had produced lesions which were very definitely those of pellagra, in one instance fulminating pellagra. Bean, Spies and Vilter ('44) have recently reported the appearance of the pellagra syndrome associated with therapeutic irradiation.

The skin lesions of Chas G., with removal of the sunburn factor, faded considerably during the first few days of hospitalization, but a residual roughness and redness remained which was unchanged during the 9 weeks on the basal diet. With administration of nicotinic amide there was a rather prompt improvement, the skin lesions becoming softer and less pigmented.

METABOLIC OBSERVATIONS

Cooperation in collection of specimens was excellent, judging from excretion of creatinine as an index of completeness: Etta R. excreted regularly about 1.0 gm. and Chas. G. about 1.4 gm. Since the four specimens used each week for analysis were usually from successive days, inequalities of collection periods were largely cancelled.

The average values for nicotinic acid and for trigonelline, on the four specimens analyzed, were taken as the daily excretions for the week. Weekly fluctuations, so calculated, are shown in the accompanying chart.

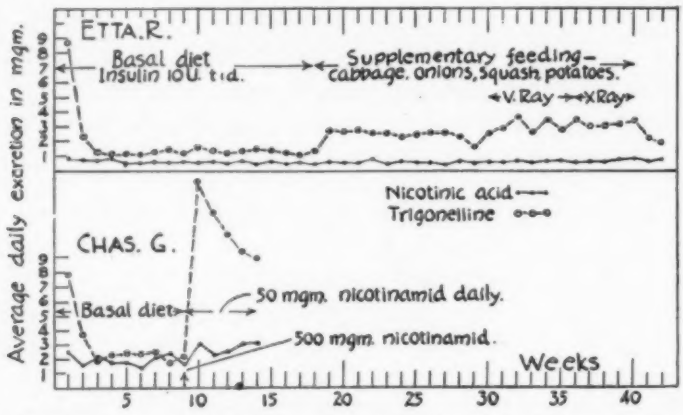

Fig. 1 Showing the average daily values for urinary excretion of nicotinic acid and trigonelline, during a study of nicotinic acid restriction.

The nicotinic acid output

The urinary excretion of Etta R. amounted to a little less than a milligram nicotinic acid daily at the start of her study, and it remained at this level to the end. This is just below the range of 1 to 3 mg. usually observed on normal controls, and might possibly be taken as an expression of her poor nutritional state. However, Chas. G., who we have reason to believe was in a somewhat more depleted state of nicotinic acid nutrition at entrance, excreted about 2 mg. daily with essentially the same intake. Also, following the test dose of 500 mg. nicotinic amide and the therapeutic administration of 50 mg. nicotinic amide daily, over a period of 5 weeks, his output was only increased to a level of about 3 mg. daily. These observations fail to show anything of value in the urinary assay of nicotinic acid as an index of the nutritional status; they are in agreement with the results of other studies in this respect (Briggs, '41; Field et al., '41; Goldsmith, '42).

The output of trigonelline

It is obvious that we have been successful in devising a diet low in trigonelline as well as nicotinic acid. The excretion of trigonelline by

Etta R. dropped within 3 weeks to about 1 mg. per day, and remained at this low level until the supplementary feedings were started; the increased output during the remainder of her study was due to preformed trigonelline in the supplements — biological assay revealed only small quantities of nicotinic acid in these foodstuffs. Chas. G. excreted a little more than 2 mg. per day during his period of restriction. Since his diet only differed from the basal diet by the supplementary 50 gm. of spaghetti plus 5 gm. of cheese, it is possible that his slightly greater output of trigonelline, was an expression of greater total metabolism.

Sarett ('42) observed, with dogs on a black tongue producing diet, that the output of trigonelline dropped progressively to zero during the course of a few months. Our failure to observe a drop below the level of the third week is no doubt due, in part, to greater quantities of nicotinic acid and trigonelline in our diets; but we also believe that the direct technique employed by us is better suited to the detection of small quantities of trigonelline than the indirect technique of Sarett.

Niacin tolerance tests

Each of the subjects, at the close of their period of dietary restriction, was given the tolerance test suggested by Sarett, Huff and Perlzweig ('42): 500 mg. of nicotinic amide was administered by mouth and the quantity of extra trigonelline plus nicotinic acid bodies excreted during the next 24 hours determined by chemical analysis. According to their observations, with a state of nicotinic acid deficiency, the "extra" excretion should be less than 25 mg. (less than 5% of the test dose).

The "extra" output of Etta R. was 76 mg., a little more than 15%; the "extra" output of Chas. G. was 38 mg., between 5 and 10% of the 500 mg. ingested. According to the Sarett et al. standard then, neither of these subjects could be called deficient. We have the feeling that this standard must indicate an advanced state of deficiency. As a matter of fact, only one of their control subjects excreted less than 17% "extra" trigonelline plus nicotinic acid; and our own observations on three subjects who only adhered to the basal diet for a short time gave the following results for "extra" output: Bryant 18.2%; Caldwell 18.3%; Higgs 14.4%. Probably, with our technique, anything less than 75 mg. "extra", or 15%, should be considered as representing deficiency. Goldsmith ('44) has recently observed that normal controls excreted more than 20 mg. "extra" within 6 hours after ingestion of only 300 mg. nicotinic amide. These results seem to be in line with our own.

The urinary assays of F_2

From the work of Huff and Perlzweig ('43) and Najjar, White and Scott ('44) it seems probable that the fluorescent substance F_2 is a methylated derivative of nicotinic acid, presumably a precursor of endogenous trigonelline.

Since F_2 has been found absent from the urine in pellagra (Najjar and Holt, '41) it has been suggested that the nutritional state with respect to nicotinic acid might be revealed by the urinary assay of F_2. From our own limited experience this seems to be true (Singal, Hall and Sydenstricker, '44); however, the value of the test has been questioned by Mickelsen ('44).

The results of the F_2 determinations are not shown on the chart for the reason that all values were zero for each of these subjects, during the early as well as the late stages of the period of restriction. F_2 did, however, appear promptly following the administration of the test doses of nicotinic amide.

It is evident, therefore, that urinary F_2 may be absent in mild states of niacin deficiency: each of these subjects had questionable skin lesions; neither subject appeared to be in a severe state of deficiency from clinical examination nor from the niacin tolerance test.

Nicotinic acid requirements

The evidence of dietary surveys suggests that the minimal niacin requirement is considerably less than the figure of 18 mg. daily which was recommended by the National Research Council. Dann ('44) has pointed out that dietary calculations from these surveys in various localities, in which neither pellagra nor gross evidence of milder deficiency were observed, indicate a nicotinic acid intake frequently as low as 5 mg. daily or less.

By analysis of duplicate diets, Winters and Leslie ('43) have found that self chosen diets of a low income group in Austin, Texas, provided a daily intake of nicotinic acid ranging from 2.7 to 9.8 mg. with a mean of 4.2 mg. The only evidence of niacin deficiency observed was some lateral redness of the tongue and red, swollen papillae.

It becomes less surprising, therefore, that Etta R., with a daily intake of about 3 mg. niacin daily over a period of 42 weeks, exhibited only lateral redness of the tongue and some increase in the pigmentation over her elbows. However, the appearance of minimal lesions in this subject, and the fact that the lesions of Chas. G. failed to heal on an intake of about 3 mg., suggests that we were working with intakes

somewhere near the minimal human requirement, probably a little below the minium requirement for the conditions of our study.

One factor which must be considered is the possibility of intestinal biosynthesis of niacin. Najjar and Holt ('43) have observed evidence of biosynthesis of thiamine; and evidence from the same laboratory (Najjar, Johns, Mediary, Fleishman and Holt '44) has just appeared, which suggests that biosynthesis of riboflavin may be sufficient for human requirements. So, an analogous intestinal biosynthesis of niacin might provide a protective supplement and complicate a metabolic study such as the present one. We have a single observation bearing on this point: Etta R. took 4 gm. of sulfaguanidine daily, during her twenty-ninth week and, as may be observed on the chart, there was a slight but definite drop in the output of trigonelline.

Each of these subjects had been treated previously, for classical pellagra. Consequently, the lack of an individual idiosyncrasy, if such a factor really exists, cannot be invoked to explain their failure to exhibit the syndrome during this study.

Except for the 50 gm. of hominy, our basal diet contained no corn. Because of the association of maize eating and clinical pellagra it has been suggested by Chick ('33) that a toxic substance in corn tends to neutralize the pellagra preventive vitamin. Possibly we have observed all which should have been expected on a corn poor diet. It is conceivable, of course, that something in corn might inhibit the intestinal biosynthesis of nicotinic acid.

SUMMARY

Two subjects, each of whom previously had been a pellagra patient, restricted themselves to a diet low in trigonelline and providing only about 3 mg. of nicotinic acid daily, one for a period of 9 weeks and the other for 42 weeks.

Each had minimal lesions of nicotinic acid deficiency at the start, but in neither was there any significant development in the direction of pellagra.

The nicotinic acid excretion of one remained low throughout the study; the other remained at a normal level.

The trigonelline output in each case dropped to a low level within 3 weeks, but showed no tendency to fall to a lower level with prolonged restriction.

Niacin tolerance tests in each case were interpreted to indicate a mild state of deficiency.

Tests for the fluorescent substance F_2 were zero at the start as well as at the close of the periods of restriction.

It is suggested that the failure in the development of pellagra may have been due possibly to intestinal biosynthesis of nicotinic acid, and possibly to the fact that the diet contained little corn.

Apparently, under the conditions of our study, the 3 mg. daily of the diet provided an intake somewhere near the minimal niacin requirement.

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THE EFFECT OF THE LEVEL OF PROTEIN IN THE DIET ON THE UTILIZATION OF VITAMIN A[1]

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This is a report upon the third phase of an experiment planned to determine the effect of various dietary factors upon the utilization of vitamin A. In the two previously published studies, it was reported by Muelder and Kelly ('41, '42) that the caloric intake was responsible for greater gains in weight than was the increased unitage of vitamin A, and that the level of fat in the diet did not significantly increase the amount of weight gained. Neither the caloric intake nor fat level affected the number of "abscesses". In the present study, the effect of the level of protein in the diet upon the utilization of vitamin A is considered.

Other investigations have indicated that the utilization of vitamin A might be affected by the level of protein although there is no work with which this study is precisely comparable. Randoin and Queuille ('34) found no effect upon the time of development of xerophthalmia in young rats on a vitamin A deficient diet with varying proportions of protein; however, cessation of growth was delayed in rats receiving 27% and 37% protein. Sampson et al. ('32) reported no appreciable alteration of the absorption of nitrogen, but an increase in nitrogen metabolism and a decrease in rate of gain in body weight per unit weight of food ingested when rats were being depleted of vitamin A. Emerique ('36) obtained similar results. Basu and De ('41) found that 18% of protein in the ration considerably increase liver storage of vitamin A over that stored on a ration containing 8% protein when both groups of rats received 100 I.U. of vitamin A daily. Baumann, Foster and Moore ('42) found that with low protein diets there was less storage of vitamin A in the liver and that vitamin A previously stored in the liver was more quickly depleted.

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EXPERIMENTAL PROCEDURE

In this investigation, triads of rats of the same sex and litter were started on the vitamin A deficient diet containing an 18% level of protein (table 1) at a weight of 50 to 55 gm. and an age of 25 to 28 days. At depletion, as determined by failure to gain weight for 1 week and evidence of xerophthalmia, the basal ration of the triads of rats was changed, each member of the triad receiving an equi-caloric basal diet containing, respectively, 9, 18 and 36% protein. Each member of the triad received the same level of vitamin A supplement,[3] 0, 1, 3 or 6 I.U. Twenty-four triads, twelve male and twelve female, were placed on each level of vitamin supplement making a total of 288 animals used in the experiment. The methods of preparation and of feeding the supple-

TABLE 1

Composition of basal rations varied with respect to source of protein.[1]

INGREDIENTS	DIET DURING DEPLETION	EXPERIMENTAL DIETS		
	%	%	%	%
Casein, vitamin A-extracted + 0.05% cystine	18	9	18	36
Cornstarch, irradiated	73	82	73	55
Fat, refined cottonseed oil	5	5	5	5
Salt mixture, Osborne and Mendel	4	4	4	4

[1] Throughout the entire experiment 0.5 gm. bakers' yeast was fed daily as a supplement to supply the B vitamins.

ments were the same as used previously (Muelder and Kelly, '41). The animals were weighed weekly and the food consumption was checked daily. The food consumption of a triad was restricted to the level of the poorest feeder in that triad.

Criteria used to measure the response of the animals to vitamin A were: growth, as shown by gain in weight and by length of rat, and numerical total of the incidence of accumulations of keratinized epithelial cells, called "abscesses". Differential leucocyte counts were made by standard procedure from blood smears of a number of animals from each group at the end of the experimental period. Measurements of the total thicknesses of the midlingual and midlabial dentin were also made as an index of the utilization of vitamin A. After death, the animals were decapitated and the heads fixed in a 4% aqueous solution of formaldehyde. Transverse sections of lower left and right incisors

[3] Reference cod liver oil (U. S. Pharmacopeia, XI).

were prepared by grinding on medium and fine carborundum stones. With a filarmicrometer eyepiece, calibrated to a stage micrometer, measurements were recorded for the narrowest widths of labial and lingual dentin within the area defined by the width of the pulp cavity and parallel to the lines of dentin tubules.

RESULTS AND DISCUSSION

The animals were depleted of vitamin A within an average of 6.3 weeks, the growth plateau occurring at an average weight of 116.2 gm. The food intake averaged 49.9 gm. per week during the depletion period.

Growth responses and food utilization

A summary of the data, expressed as combined averages for the different levels of protein fed, for the food intakes and weight changes, together with the length of rats and number of "abscesses" evident at autopsy is given in table 2. Statistical analyses of the means were made using the formula "t" $= \frac{M - M_1}{\frac{(\sigma)^2}{n} \quad \frac{(\sigma_1)^2}{n_1}}$. From these analyses, it was found that the level of protein had no very significant effect upon gains in weight on any level of vitamin A. A slightly significant gain in

TABLE 2

Food intake, weight changes, and autopsy findings expressed as combined averages for males and females.

NO. OF ANIMALS	LEVEL OF VITAMIN A PER DAY	LEVEL OF PROTEIN	AV. WEEKLY FOOD INTAKE	AV. WEEKLY CHANGE IN WEIGHT	GM. GAIN Gm. food intake	"ABSCESSES" EVIDENCE AT AUTOPSY	LENGTH OF RAT AT AUTOPSY
	I.U.	%	*gm.*	*gm.*		*number*	*inches*
24		9	23.93 ± .97 [1]	− 9.61 ± .85		4.88 ± .25	12.98 [2]
24	0	18	22.97 ± .96	− 8.11 ± .84		5.08 ± .27	12.93
24		36	23.66 ± .90	− 9.39 ± .86		4.96 ± .29	12.50
24		9	34.92 ± .62	+ .007 ± .43	.0002	2.21 ± .33	12.95 ± .13
24	1	18	34.77 ± .60	+ .96 ± .48	.0259	1.62 ± .25	12.88 ± .18
24		36	34.76 ± .60	+ .35 ± .47	.0107	1.71 ± .30	12.91 ± .14
24		9	44.99 ± .82	+ 4.15 ± .47	.092	1.29 ± .27	13.17 ± .17
24	3	18	45.05 ± .81	+ 5.80 ± .57	.129	1.38 ± .23	13.29 ± .19
24		36	45.01 ± .82	+ 5.18 ± .53	.115	1.25 ± .26	13.29 ± .18
24		9	41.04 ± .80	+ 3.58 ± .47	.087	1.71 ± .27	13.38 ± .12
24	6	18	41.00 ± .79	+ 4.74 ± .53	.116	1.58 ± .35	13.40 ± .13
24		36	41.04 ± .80	+ 3.56 ± .47	.087	1.67 ± .22	13.12 ± .18

[1] Arithmetic mean ± standard error.

[2] Too few animals were measured before death to warrant statistical analysis of data.

weight in favor of the 18% protein level over the other 9 and 36% levels was shown at each unitage of vitamin A. As might be expected, significant gains in weight were shown on all levels of protein as the unitage of vitamin A was increased up to 3 I.U. per day. These results confirm previous findings that the level of vitamin A in the diet is an important factor in promoting gains in weight, and that vitamin A is able to function to stimulate growth when combined with diets containing a wide range of protein level.

The averages in table 2 represent gains in weight of both male and female animals. In separating the results according to sex, it was found that when either 3 or 6 I.U. of vitamin A were given, the males showed significantly greater gains than the females on each level of protein. The difference between the gain in weight of the sexes agrees with the findings of Palmer and Kennedy ('31) and Coward et al. ('31). There were no significant differences favoring one level of protein for either sex.

Since accurate measurements of the length of all the rats could not be made, the data were not analyzed statistically. The few figures obtained do not show any variation in length of the animals on the various levels of protein. Rats on a higher level of vitamin A were slightly longer.

The administration of even 1 I.U. of vitamin A produced a highly significant reduction in the number of "abscesses" over those occurring in the animals receiving none. Three and 6 I.U. brought no further significant reduction, except a slight decrease in favor of 3 I.U. over 1 I.U. on the 9% level of protein. Some pathological lesions were present after 6 weeks on a diet containing as much as 6 I.U. of vitamin D daily, which agrees with the observations reported by Richards and Simpson ('34).

Use of differential leucocyte counts as a criterion for utilization of vitamin A

Some investigators have indicated that differential leucocyte counts were altered in vitamin A deficiency states. The data presented in table 3 were collected in an effort to determine whether or not the blood picture would serve as a satisfactory criterion for the utilization of vitamin A under the conditions of this experiment. The small number of blood smears made from animals receiving no vitamin A is due to the impossibility of obtaining satisfactory blood samples because of unpredictable times of death among these groups. Total leucocyte counts probably fall within the normal limits for rats in all instances,

although information on normal animals is limited and varies a great deal. The few data from animals receiving no vitamin A indicated total leucocyte counts almost twice as high as those in other animals. It would appear that the protein levels of the diets used in this study had no effect on the total or differential leucocyte counts. There is, however, an indication of increased polymorphonuclear cells among the animals receiving no vitamin A and those receiving only 1 I.U. of vitamin A daily. The possible disturbance of polymorphonuclear cells which has been noticed here agrees with the findings of Abbott and Ahmann ('38) and of Turner and Loew ('31); on the other hand, Sure, Kik and Walker ('31) found no evidence of change in the differential leucocyte count in vitamin A deficiency. Since the techniques for carrying out differential leucocyte counts are standardized and easily done, it would appear that this criterion for judging utilization would warrant further study with larger numbers of animals.

TABLE 3

Summary of differential leucocyte counts.

NUMBER OF RATS FOR TOTAL COUNTS	LEVEL OF PROTEIN IN FOOD	LEVEL OF VITAMIN A IN FOOD PER DAY	TOTAL LEUCOCYTE COUNT	DIFFERENTIAL COUNTS					
				Polymorphonuclear cells	Stabs	Large lymphocytes	Small lymphocytes	Myelocytes	Monocytes
	%	*I.U.*	*M/cmm.*	%	%	%	%	%	%
3	9	0	18.2	31	6	7	56	0	0
3	18	0	16.0	16	1	7	68	1	7
2	36	0	16.0	14	15	13	49	7	2
7	9	1	7.4	23	3	11	58	5	1
7	18	1	8.1	23	3	8	62	3	1
7	36	1	7.5	12	3	12	67	4	2
8	9	3	9.6	12	5	13	66	3	1
8	18	3	7.3	15	4	10	67	3	1
8	36	3	8.0	8	4	14	69	4	1
9	9	6	9.3	12	4	12	69	2	1
10	18	6	8.5	9	5	12	70	2	2
9	36	6	11.4	9	5	8	76	2	0

Use of dentin widths in transverse ground sections of incisors of rats as a criterion for utilization of vitamin A

A study of the ratios of midlingual to midlabial dentin-widths in transverse ground sections of lower incisors of some of the experimental

animals was made. Sections of incisors from rats receiving no vitamin A, and many of those from animals receiving 1 I.U. or even more vitamin A daily showed increased thickness of enamel-covered dentin, narrow cementum covered dentin, and irregular pulpal outline. All of these distortions of the normal growth pattern are characteristic of vitamin A deficiency, as described by Schour, Hoffman, and Smith ('41). The ratio of midlingual to midlabial dentin in the normal rat incisor is 1:1.

The ratios of lingual to labial dentin furnish little if any basis for determining the effect of protein on the utilization of vitamin A. Animals receiving no vitamin A or 1 or 3 I.U. of vitamin A showed dentin ratios of approximately 1:2 at all levels of protein except in one case. In the group receiving 3 I.U. of vitamin A and 18% protein, improvement in the condition of the teeth as shown by a ratio approximately 1:1 was observed. This small group of 4 animals does not justify the conclusion that the 18% level of protein allowed better utilization of vitamin A than 9% and 36% protein. It was not until 6 I.U. of vitamin A were included in the diet that ratios of dentin widths approached the normal relationship. These ratios were approximately 1:1 regardless of protein intake.

SUMMARY

The effect of 9, 18, or 36% of protein in the diet on the utilization of vitamin A was studied in 288 rats. The level of protein had little effect upon the utilization of vitamin A as judged by weight gains, length of the rat, and incidence of foci of keratinized epithelium. The level of vitamin A intake was directly related to rate of gain up to the level of 3 I.U. per day regardless of the level of protein in the diet. There were slightly greater gains in weight on the 18% level of protein regardless of the level of vitamin A than on other quantities of protein.

Increase in polymorphonuclear cells was observed on low vitamin A intakes.

The teeth did not show a normal midlingual to midlabial dentin ratio until 6 I.U. of vitamin A were present in the diet.

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THE NUTRITIVE VALUE OF THE FATTY ACIDS OF BUTTER INCLUDING THEIR EFFECT ON THE UTILIZATION OF CAROTENE[1]

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TWO FIGURES

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Most of the current studies of the comparative nutritive value of various fats have relied on the effect on growth as the sole criterion and have dealt with the whole fat rather than with its respective fatty acids. There is need for further information on the special physiological functions of specific acids.

That highly unsaturated fatty acids are essential in the diet has been established (Burr and Burr, '29; McAmis, Anderson, and Mendel, '29; Burr and Burr, '30; Evans and Lepkovsky, '32). The acids which will produce some curative effects in rats suffering from deficiency of fat in the diet are linoleic, linolenic, arachidonic, decosahexenoic and hexahydroxystearic (Burr and Barnes, '43).

The growth-promoting value of fatty acids of different degrees of saturation is still under investigation. Boutwell, Geyer, Elvehjem and Hart ('41) found that the saturated fatty acids of butter and especially the unsaturated acids which had been artificially saturated, when mixed as glycerides with corn oil and incorporated in a skimmed milk diet, produced better growth in rats than the unsaturated acids or butter fat itself. On the other hand, the work of Loosli, Lingenfelter, Thomas and Maynard ('44) suggests that unsaturated acids, as found in corn oil, may have a particular value for the growth of suckling rats.

The following is a report of a preliminary study of the nutritive value of some fractions of the fatty acids of butter. We have found that rats receiving the unsaturated acids of butter as the major source of fat in a normal diet, supplemented with raw carrot and Drisdol (a source of vitamin D), store more vitamin A in their livers than rats

[1] This work was supported by funds granted by the National Dairy Council on behalf of the American Dairy Association.

receiving the saturated or volatile acids. The diets containing the unsaturated acids also seem to be more efficient in producing growth, probably due to better absorption.

PROCEDURE

1. The preparation of fatty acids

Dry butter fat was saponified with 10% alcoholic potash by refluxing over steam for 2 to 3 hours. Most of the alcohol was removed by distillation and the remainder by suction over warm water. The soap was mashed with a little water and acidified, with cooling, by an excess of dilute sulphuric acid. Steam distillation was carried out until about 5 liters of distillate had been collected. This was extracted with ether and the extract was distilled to remove most of the ether. The concentrated solution was made to volume and titrated to determine the amount of acid as butyric.

The residue containing the non-volatile fatty acids was extracted with distilled ether. After the extract had been washed and dried, the ether was removed and the fatty acids were separated into the "solid" and "liquid" groups by use of the lead salt separation. Each group of acids was then dissolved in ether, washed, dried, freed from ether and refluxed with methyl alcohol containing 1 to 2% dry hydrogen chloride gas. The methyl esters so obtained were taken up in ether, washed with water, dilute sodium carbonate, and again with water. They were then freed from all water and ether and subjected to fractional distillation. The apparatus used was patterned after that of Longenecker ('37). Three fractions were taken off, the middle one being redistilled and added to the other two in such a way as to obtain finally two fractions of equal weight, having average molecular weights as different as possible. Each fraction was then saponified with alcoholic potash, freed from alcohol, acidified with an excess of dilute sulphuric acid and extracted with ether. The ether extracts were washed, dried, and the ether removed. Constants were determined on all fractions which were then stored under nitrogen at 0°C.

The average titration equivalents obtained for the high and low molecular weight liquid portions were 279 and 226; those for the high and low molecular weight solid portions were 271 and 248. The average iodine values for the high and low molecular weight liquid portions were 98 and 35; those for the high and low molecular weight solid portions were 7.8 and 0.6.

2. *The diet*

The diet used was that known as the Sherman B (Sherman and Campbell, '24), modified for the purposes of our experiment. Each 100 gm. contained 10 gm. of the test fat or fatty acid, 23.3 gm. of skimmed milk powder, and 66.6 gm. of whole wheat flour. Sodium chloride was added in amounts equal to 2% of the weight of the flour. This made the amount of test fat in the diet 9.9%. Small batches of the diets were prepared every few days and stored in the refrigerator. In the first two experiments the solid acids were melted and poured over the rest of the mixture, the whole mass being pulverized in a mortar. In experiment 3 they were first pulverized and then put through a sieve with the rest of the ingredients. The butter diet was made with whole butter.

An exception was made in the case of the diet containing the volatile acids. The 100 ml. of ether solution of these acids obtained from each pound of butter contained about 19 gm., calculated as butyric acid. This amount was added to 300 gm. of the basal diet in the small portions required for each day's feeding. The fat content of this diet was about 6%.

Six test preparations were used. The diets containing them will be designated butter, volatile, low liquid, high liquid, low solid, high solid. In addition to the diets the rats received 1 drop of Drisdol (crystalline vitamin D from ergosterol in propylene glycol) and 3 gm. of raw carrot per week. They were fed ad libitum.

3. *The experiments*

Three duplicate experiments were run, except that the first was continued for 3 weeks, and the next two for 5 weeks. Each experiment used twenty-four male rats, put on the diets at 21 days or after, and distributed by litter and weight as fairly as possible. They were kept in individual cages. The average starting weights in the three experiments ranged from 44.0 to 46.1, 45.1 to 46.1 and 34.8 to 35.0 gm.

The rats were weighed weekly and their food consumption per week was determined. In experiment 3 the feces were collected each day during the final 2 weeks on the diets and stored in the refrigerator.

The rats were killed by illuminating gas. Autopsies were performed for the purpose of noticing any gross abnormalities. The livers were saved for analyses of vitamin A and many other organs were weighed.

4. *Methods of analysis*

a. Vitamin A. The method used for extraction of vitamin A from the liver has been described elsewhere (McCoord and Luce-Clausen, '34). The vitamin A content of the petroleum ether extracts was determined by means of the Evelyn photoelectric colorimeter (Clausen et al., '42). The results are expressed in terms of International Units (I.U.), one of our blue units being equal to 3.8 I.U.

b. Lipids of the liver. A composite of half the liver tissue of each rat in a group, from experiment 3, was used for the analysis. This was enough for single determinations only. The tissue was refluxed over steam with 5% aqueous sodium hydroxide, and acidified with dilute sulphuric acid. The extraction was made first with ether and then, after removing the ether, with petroleum ether. One volume of the petroleum ether extract was shaken in a separator with two volumes of 50% alcohol containing potassium hydroxide. This made it possible to separate the fatty acids as soap from the unsaponifiable material in the petroleum ether layer. The latter was drawn off into a small suction flask, the petroleum ether sucked off, and the flask weighed. The other portion was heated on the steam bath to remove most of the alcohol and was then acidified with dilute sulphuric acid. Extraction was done with petroleum ether, the extract washed with water, and the petroleum ether removed by use of the small suction flask which was then weighed.

c. Lipids of the feces. Two 5-gm. samples of the feces from each of three rats in a group (experiment 3 only) were pulverized and refluxed on the steam bath with 10% alcoholic caustic soda. They were diluted with 3 parts of water, containing an excess of sulphuric acid, and extracted, first with ether, and then after the removal of ether, with petroleum ether. The latter extract was washed with water and the petroleum ether layer was taken off into a small suction flask. The solvent was removed and the flask weighed. In most cases the lipid material from the duplicate samples was combined before being used for the determination of iodine number.

d. Constants. Iodine number was determined by the method of Wijs. The usual saponification method was followed whether the material to be tested was a fat or a fatty acid, but in the latter case we use the term "titration equivalent" instead of "saponification equivalent".

RESULTS

1. *Growth*

A study of the gains in weight made in relation to the amounts of food eaten suggests a superiority for the unsaturated acids. This relation

was studied by determining the per cent efficiency of the diet in the following manner. The ratio of the gain in weight over the food consumption for each rat while on the diet was determined and multiplied by 100. The average of the values so obtained for the four rats of a group is reported as the efficiency of that diet in an experiment. As shown in figure 1 the efficiency values for the low liquid diet, in all three experiments, are at the top. They are 46.8%, 42.3% and 41.0%, respectively. The corresponding values for the high liquid diets are 44.9%,

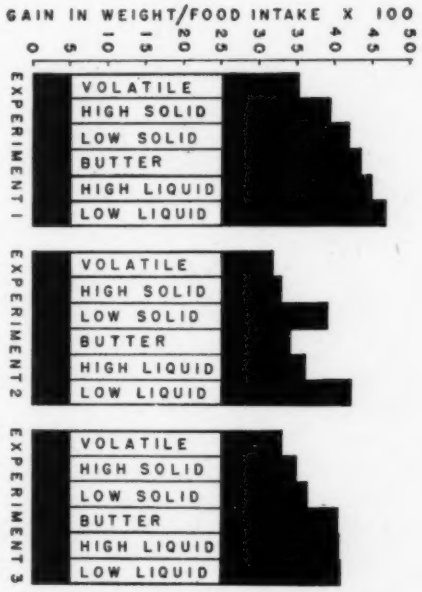
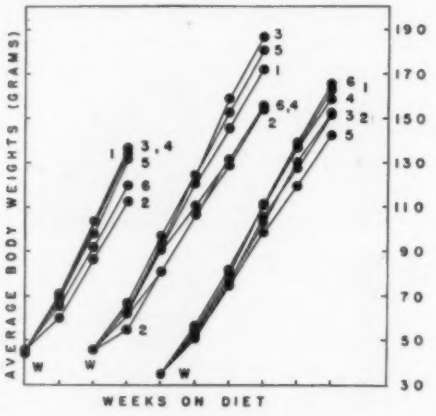


Fig. 1 The efficiency of the diet is determined by dividing the total gain in weight of each rat while on the test diet by the total amount of food eaten and multiplying by 100. Each column represents the average values for four male rats.

36.2% and 40.7% which means that in the first two experiments the high liquid diets were inferior to the low liquid ones. Also in those experiments the values for butter, 43.4% and 34.2%, are still lower while in the third experiment the value of 40.6% is practically identical with those of the liquid diets. With the exception of the low solid group of experiment 2 with a value of 39.2% there is a progressive decrease in values from butter to low solid to high solid to volatile. The repetition of this order throughout three experiments probably means that there

is a real superiority of the liquid and butter diets over the solid and volatile diets, with the possible exception of the low solid diet. These results are probably not related to the amount of vitamin A available inasmuch as the rats receiving the butter diet were getting much more of the vitamin than any of the others and yet their growth was not outstanding.

The growth curves, figure 2, drawn without any reference to the amounts of food eaten do not give clear evidence for the superiority



Growth curves

Fig. 2 These curves represent the results for experiments 1, 2, and 3, respectively. Body weights are plotted against the number of weeks on the test diets. Each spot is the average weight of four male rats. W indicates the average weaning weight. The abscissa is marked off in intervals, each indicating 1 week. The curves for the different diets are numbered to indicate: 1 — butter diet; 2 — volatile acid diet; 3 — low liquid diet; 4 — high liquid diet; 5 — low solid diet; 6 — high solid diet.

of the unsaturated fatty acids. Evidently this might have been demonstrated by such curves if the food intake of the rats had been standardized, instead of being unrestricted. The butter groups were among the best growing in each experiment and the volatile acids groups were among the poorest growing. Any other conclusions would require proving through the use of large numbers of animals.

2. Storage of vitamin A in the liver

The diets containing the liquid fatty acids appear to have been superior also in helping the rats to store vitamin A in their livers, when they were being fed raw carrot as its source. In table 1 the total

amounts of vitamin A stored in the livers are recorded, as averages for the four rats in the group. The values for the animals receiving butter are very high compared to the rest, as would be expected from the fact that they were getting an added source of the vitamin in the butter. Of the groups which had the 3-gm. raw carrot per week as its source, those on the liquid diets stored the most vitamin A. The average values for the six groups on the liquid diets were higher than any of the average values for the six groups on the solid acid diets. There was overlapping, however, of individual results. The amounts of vitamin A stored in the livers of the groups receiving the volatile acids were very low, probably in part because the rats tended to have diarrhea.

TABLE 1

Vitamin A in the livers of rats fed the diets specified.

Each figure is the average value in international units for 4 male rats. Concentration is expressed as units vitamin A per 100 gm. wet tissue. Standard deviations are included.

	EXPERIMENT I		EXPERIMENT II		EXPERIMENT III	
	Total	Concentration	Total	Concentration	Total	Concentration
Butter	444.7 ± 62.5	7540 ± 816	457.2 ± 72.5	6678 ± 1459	377.3 ± 29.8	5203 ± 506
Volatile	30.9 ± 12.4	709 ± 366	44.1 ± 7.0	718 ± 122	32.3 ± 6.1	477 ± 102
Low liquid	141.1 ± 41.3	2235 ± 537	125.5 ± 34.2	1415 ± 420	135.5 ± 64.2	1956 ± 1102
High liquid	139.4 ± 45.4	2206 ± 716	133.2 ± 53.3	2119 ± 1179	152.7 ± 44.0	1893 ± 505
Low solid	66.0 ± 9.8	1247 ± 91	71.1 ± 27.9	1027 ± 433	86.7 ± 26.8	1193 ± 196
High solid	75.8 ± 23.2	1585 ± 621	68.4 ± 19.4	1207 ± 660	88.0 ± 22.9	1161 ± 305

Almost the same conclusions can be drawn from a study of the concentration of vitamin A in the livers, also reported in table 1. The values for the butter groups are very high and for the volatile acids groups are very low. With one exception the average values for the groups on the liquid acid diets are higher than those for the solid acid diets.

For statistical confirmation of this conclusion that there was a real difference in the total amount of storage of vitamin A in the livers, depending upon whether the diet contained the liquid or the solid acids, we made comparisons of homogeneity between various groups. As the Snedecor F values for the three low liquid groups lay within the 5% point these groups were combined. Similarly, combinations of the low solid, of the high liquid, and of the high solid groups proved to be justified. The same result was obtained when we considered the combination of the low liquid with the high liquid groups, and of the low solid with the high solid ones. However, a Snedecor F value of 30 (1%

point is 7.2) found for a combination of all 48 vitamin A values satisfied us that there is a real difference in the amount of storage between the liquid and the solid groups.

3. Lipids of the liver

No gross difference in the lipid composition of the liver from the different groups was observed. As seen in table 2 the values for the petroleum ether soluble unsaponifiable material were fairly constant. Since only one determination was made on a composite sample from each group no significance can be attached to the exception found in the case of the high liquid group.

TABLE 2

Lipid composition of livers of rats on following diets.

	BUTTER	VOLATILE	LOW LIQUID	HIGH LIQUID	LOW SOLID	HIGH SOLID
Unsaponifiable (gm./liver)	0.024	0.026	0.024	0.047	0.023	0.029
Total fatty acids (gm./liver)	0.16	0.21	0.21	0.17	0.19	0.18
Iodine value of fatty acids	128.6	113.7	108.8	106.9	105.6	115.2

Neither was there any great variation in the total amount of fatty acids per liver. However, the iodine value of the fatty acids of the livers from the butter group was 128.6 which is definitely higher than that of any of the other groups whose iodine numbers ranged from 105.6 to 115.2

4. Lipids of the feces

In the final column of table 3 we have listed, under the heading of per cent absorption the percentage of the test material ingested which was not recovered in the feces. We assume that the portion not recovered in the feces was absorbed but it is not necessarily true that the portion recovered in the feces represents unabsorbed material. In fact, in the butter, volatile, low liquid, and high liquid groups the fatty acids fed were probably completely absorbed, the fat obtained in the feces being excretory. We base this conviction on the facts that only small amounts of fecal fatty acids were obtained, and that the values were similar for the different diets, as were the iodine numbers. For these groups the percentage absorption ranged between 89 and 97, and the iodine values between 52.4 and 79.0.

In the low solid group the average percentage absorption for the three animals studied is 71.3, that of the high solid groups 42.2. These

acids were not so well absorbed as the liquid acids and there is a difference in absorption between the low and high solid groups, probably due to an increased concentration of long chain acids in the latter. The molecular weights of the low and high solid acids fed averaged about 248 and 271, those of the fatty acids obtained in the feces averaged

TABLE 3

Absorption of fat during final 2 weeks on the diets.

DIET	RAT NO.	DRY WEIGHT FECES	FATTY ACID	TOTAL FATTY ACID EXCRETED	IODINE VALUE	ABSORPTION
		gm.	%	*gm.*		%
Butter	49	12.2	5.6	0.68	52.4	95.2
Butter	51	11.7	6.6	0.77	53.7	93.2
Butter	52	14.1 Av.=12.7	7.0 Av.=6.4	0.99 Av.=0.81	67.4	90.7 Av.=93.0
Volatile	53 [1]	12.0	5.2	0.62	65.4	92.0
Volatile	55 [1]	13.9 Av.=12.9	6.3 Av.=5.7	0.87 Av.=0.75	70.5	89.2 Av.=90.6
Low liquid	57	14.2	5.3	0.75	71.5	95.4
Low liquid	58	10.1	4.4	0.44	55.1	96.6
Low liquid	60	10.4 Av.=11.5	7.9 Av.=5.9	0.82 Av.=0.67	64.1	93.1 Av.=95.0
High liquid	61	13.5	6.7	0.91	77.3	93.0
High liquid	62	14.4	6.7	0.97	79.0	93.0
High liquid	64	13.8 Av.=13.9	6.6 Av.=6.7	0.91 Av.=0.93	73.4	93.8 Av.=93.3
Low solid	65	17.4	19.0	3.31	14.9	77.9
Low solid	66	25.3	28.4	7.18	12.8	55.2
Low solid	67	11.4 Av.=18.0	18.4 Av.=21.9	2.10 Av.=4.20	18.5	80.8 Av.=71.3
High solid	69	24.4	35.4	8.65	13.5	43.4
High solid	70	25.4	36.1	9.17	13.9	46.7
High solid	71	28.1 Av.=26.0	39.4 Av.=37.0	11.09 Av.=9.64	12.5	36.5 Av.=42.2

[1] Rats were not having diarrhea during this time.

261 and 282. It appears then that either selective absorption or selective excretion occurred, with a tendency to get rid of the longer chain acids. The melting points, on the other hand, of the mixtures fed and of those obtained did not vary much from 55°C. The average iodine value of the low solid acids increased from 0.6 for those fed to 15.4 for those obtained from the feces. That of the high solid acids fed was 7.8 and for the fatty acids obtained 13.5.

DISCUSSION

The greater efficiency of the unsaturated acids in producing gains in weight has been reported by other workers. In 1927 Ozaki studied the comparative gains in weight made by rats being fed various fatty acids

as 10% of the diet and reported the following order: oleic 39.0 gm., palmitic 26.0 gm., stearic 12.5 gm., myristic 6.0 gm., and lauric 0.5 gm. Loosli et al. ('44) studied the growth of suckling rats as influenced by feeding to the mothers at parturition an ether-extracted purified diet supplemented with test fats. No increase in the weight of the young could be produced by adding ethyl linolate to the basal diet, nor by adding 10% hydrogenated corn oil or hydrogenated coconut oil, even though the latter was known to have been well absorbed. But the use of 10% corn oil, which is a good source of oleic acid as well as of linoleic, caused an increase in the average weight of the litters at 17 days from 110.6 to 129.1 gm. There thus appeared to be an advantage of unsaturated fatty acids, other than that of supplying linoleic acid. We assume that our diets, inasmuch as they contained two-thirds whole wheat flour, were adequate with respect to this acid, and yet those containing the liquid acids, particularly of lower molecular weight, tended to be more efficient in causing gains in weight than those containing the solid acids.

Much of the difference in efficiency between the liquid and solid acid diets can be explained by the poorer absorption of the solid acids. For instance, assuming 90% absorption of the non-fat solids and a constancy of the values of table 3 for fat absorption, and recalculating the efficiency by the formula $\frac{\text{gain in weight}}{\text{calories of absorbed food}} \times 100$, the following results were obtained for experiment 3: low liquid 10.1, high liquid 10.3, low solid 9.4 and high solid 9.7.

Also, we found an improved utilization of the carotene from carrot in the rats receiving the unsaturated acids. This does not seem to have depended on the molecular weights of the acids as the high and low groups produced the same results. Nor does the fact that there was a wide divergence in the degree of unsaturation of the two groups of unsaturated acids seem to have made any difference. We must conclude then that the effective acid or acids occurred in both liquid acid diets in adequate amounts. Since corn oil is a particularly good source of unsaturated acids it may be that its value in the experiments of Loosli et al. ('44) consisted in making more available the carotene fed in hydrogenated coconut oil. They do not report observations of the vitamin A content of the livers of the young.

The possibility of vitamin A or carotene of the butter having been carried over with the fractions of unsaturated acids has been considered. It does not seem possible, however, that the vitamin could withstand the chemical processes used or the distillation at 3 mm. pressure. Tests on the preparations with antimony trichloride gave either a zero

or very slight reading in the Evelyn photoelectric colorimeter which did not differ between the liquid and the solid fractions.

The smaller storage of vitamin A in the livers of our rats fed the diets containing the solid acids might be attributed entirely to poorer absorption of these acids than of the liquid acids. Some of the carotene might have been dissolved in the unabsorbed acids and carried out with them as fat soluble vitamins are known to be carried out by mineral oil. If that were true, however, we should expect the amount of absorption of the vitamin to parallel the amount of absorption of the fat, and this is not the case. The amounts of vitamin A stored in the livers of the rats ingesting the low solid acids were practically identical with those of the rats fed the high solid acids and yet there was a difference in the amount of absorption of 29%, the low solids being 71.3% absorbed and the high solids 42.2%.

The question of how much fat in the diet is necessary for optimal utilization of the carotene from carrot has not been settled, nor do we know how much the nature of the fat influences the degree of absorption. It would appear that the food absorbed from the high solid diet had a fat content of at least 5%. This is calculated on the basis of a fat content of the flour of 1.9% and an absorption of the high solid acids of 42.2%. A much greater amount of fat was absorbed from the low solid diet.

CONCLUSIONS

When the fatty acids of butter were obtained in five fractions designated as volatile, low molecular weight liquid, high molecular weight liquid, low molecular weight solid and high molecular weight solid, and substituted for the milk fat in a normal diet, the following results were observed:

1. The efficiency of the butter diet in producing gains in body weight was either matched or improved upon by the liquid acid diets. With the exception of one group out of six the solid acid diets were inferior to the butter diet in this respect, due largely in all probability to poorer absorption of the solid acids. The diet containing the volatile acids was the poorest. When the results on growth were studied without reference to the amounts of food eaten, then the liquid fractions did not show a consistent superiority over the solid ones.

2. The liquid acids were absorbed very well but the average absorption of the low solid acids was 71.3%, and of the high solid acids only 42.2%.

3. The lipid composition of the livers studied showed no real differences.

4. The nature of the fat being absorbed seemed to be of importance in the utilization of carotene from carrot by the rat. The amount of storage of vitamin A in the livers of the rats fed the liquid acid diets was greater than that in the livers of the rats fed the solid acid diets. In the six liquid groups the average amounts of vitamin A stored varied from 125.5 to 152.7 I.U. Corresponding values for the six solid groups ranged from 66.0 to 88.0 I.U.

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THE EFFECT OF ATABRINE ON THIAMINE DEFICIENCY IN THE YOUNG RAT[1]

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ONE FIGURE

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Previous studies on the relation of nutrition to the tolerance of atabrine by rats (Hegsted, McKibbin and Stare, '44a) led to the demonstration of a "choline-like" or "choline sparing" action of atabrine (Hegsted, McKibbin and Stare, '44b). Young rats receiving 40 mg. % of atabrine in the diet not only survived the acute stage of choline deficiency, but did not develop the hemorrhagic kidneys associated with this deficiency syndrome. This paper reports further experiments which demonstrate that atabrine has a "thiamine sparing" action.

EXPERIMENTAL[2] AND RESULTS

Thirty-six young male rats were fed a purified ration low in thiamine, half of the animals receiving the same diet plus 40 mg. of atabrine hydrochloride per 100 gm. of ration. The composition of the ration was the same as used in previous experiments (Hegsted, McKibbin and Stare, '44a) except that thiamine was lacking from the vitamin mixture. After 13 days the animals not receiving atabrine began to lose weight; they were then divided into six groups and fed thiamine supplements. The pertinent results are presented in table 1. It appeared that the rats receiving atabrine made much more efficient use of low levels of thiamine. However, the rations had been fed ad libitum and the animals not receiving the drug had gained somewhat faster and had lost a few grams in weight when supplements were started. Those receiving atabrine were still gaining. In order to rule out the effect of food consumption,

[1] The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Harvard University.

[2] We are indebted to Merck and Company, Rahway, New Jersey, for furnishing the crystalline-B-vitamins and the alpha-tocopherol, and to Abbott Laboratories, North Chicago, Illinois, for furnishing Haliver Oil.

TABLE 1

The effect of atabrine on the response to various levels of thiamine.

GROUP	ATABRINE IN RATION	WEIGHT AT END OF DEPLETION PERIOD	THIAMINE SUPPLEMENT	GAIN IN 30 DAYS	GAIN OF ATABRINE GROUPS COMPARED TO CONTROL
	mg. %	*gm.*	*µg. per day*	*gm.*	%
1	0	17.0	3	7.5	
2	40	18.5	3	23.8	318
3	0	18.5	6	36.0	
4	40	16.0	6	50.0	139
5	0	24.8	9	60.4	
6	40	19.2	9	54.4	90

the experiment was repeated and the food intake of the control animals limited to that consumed by the atabrine groups until their food intake fell below that of those receiving atabrine. After 21 days, the animals not receiving atabrine had lost considerable weight and were given thiamine supplements. Animals receiving atabrine, however, were continued on the basal ration without supplementation for 8 days longer. In spite of the longer depletion period, the atabrine groups gained more (1.78 gm. per day on 3 µg., and 2.36 gm. per day on 6 µg. per day) than

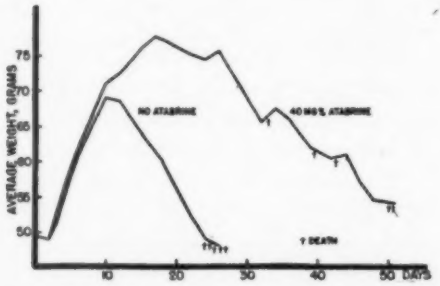

Fig. 1 The effect of atabrine on the growth of rats deficient in thiamine.

the controls on the same levels of thiamine supplementation (1.20 gm. per day on 3 µg., and 2.26 on 6 µg. per day).

In a third experiment comparable groups of animals on the basal ration and ration plus atabrine were allowed to continue until death. As in the second experiment, the food intake was equalized until the tenth day when the intake of the controls fell below that of the atabrine group. The average growth curve for the animals and time of death are shown in figure 1. It is apparent that the course of the deficiency was much more acute in the animals not receiving atabrine. They lived an average

of 25 days, whereas those animals receiving atabrine lived on the average of 43 days. Severe symptoms of polyneuritis were observed in most of the animals receiving atabrine while the controls usually died before showing clear-cut symptoms.

Thus it appears that regardless of the criteria used as evidence of deficiency (loss of weight, response to supplements, time of death), atabrine exerts a "thiamine sparing" action in young rats fed a ration low in thiamine. It will be noted in table 1 that this effect is more pronounced at the lowest level of thiamine intake, less so at the 6 μg. intake, and absent at the highest level of thiamine supplementation. This would be expected since it has been shown (Hegsted, McKibbin and Stare, '44a) that on a normal ration this level of atabrine inhibits growth.

SUMMARY

The addition of atabrine to a thiamine deficient diet for rats delays loss of weight, onset of symptoms, and death. Low levels of thiamine produce greater gains in animals receiving atabrine than in controls. Atabrine thus has a "thiamine sparing" action.

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